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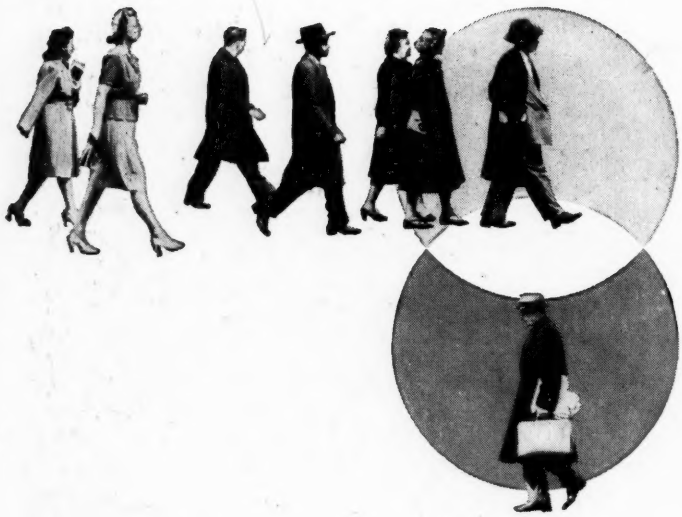

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The Journal of Allergy

VOL. 25 MAY, 1954 NO. 3

Original Articles

SUPPRESSION BY CORTISONE OF GRANULOMA FORMATION AND ANTIBODY IN GUINEA PIGS RECEIVING EGG ALBUMIN WITH FREUND ADJUVANTS

EDWARD E. FISCHEL, M.D., ELVIN A. KABAT, PH.D., HERBERT C. STOERK, M.D., MARVIN SKOLNICK, M.D., AND ADA E. BEZER, M.D., NEW YORK, N. Y.

CORTISONE and corticotropin have been found to inhibit antibody formation in rabbits after repeated injection of antigen intracutaneously or intravenously.[1, 2] The inhibition is manifested when cortisone is administered during the primary response, after immunization is well established, and still later on elicitation of the secondary response.[3] A striking diminution of lymphoid tissue and disruption of the normal architecture of the lymphoid organs are associated with the administration of adrenal cortical hormone.[4, 5]

The development of acute disseminated encephalomyelitis in animals receiving homologous brain emulsions with Freund adjuvants has been inhibited with corticotropin[6] and cortisone.[7] In the latter study with monkeys receiving large doses of cortisone, the suppression of encephalomyelitis correlated strikingly with the suppression at the inoculation sites of the granulomatous response to the dead tubercle bacilli contained in the antigen-adjuvant emulsion.[7] A suppressive effect of cortisone on antibody formation in guinea pigs injected with egg albumin in the Freund adjuvants was reported by Germuth and associates.[8]

Antibody production with the use of adjuvants depends in large part on inflammation and granuloma formation at the site of antigen-adjuvant depot.[9-11] When a potent stimulus, dead tubercle bacilli, is omitted from the antigen-adjuvant mixture, granuloma formation does not occur and, concurrently, antibody formation in guinea pigs is markedly diminished.[12, 13]

The purpose of this study was to determine the effect of cortisone at two dosage levels on antibody production in guinea pigs, when the antigen is administered with the Freund adjuvants. Antibody formation was found to be

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This work was supported by a grant (B-9) from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, United States Public Health Service, and in part by the Helen Hay Whitney Foundation, the Masonic Foundation for Medical Research and Human Welfare, and the William J. Matheson Commission.

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markedly suppressed by the larger dose of cortisone and this was accompanied by the absence of local or systemic granuloma formation.

EXPERIMENT

Four-times-recrystallized egg albumin was used as antigen. The antigen-adjuvant emulsion was made by mixing the ingredients in a mortar in the following proportion: 500 mg. egg albumin dissolved in 25 ml. saline, 25 ml. Aquaphor, and 50 ml. of paraffin oil containing 64 mg. of dried heat-killed tubercle bacilli. Guinea pigs weighing between 400 to 600 grams were distributed according to sex and weight in an attempt to achieve comparable control and cortisone-treated groups. Three injections of 1 ml. of antigen-adjuvant mixture were given intramuscularly at weekly intervals at different sites in the following order: right shoulder girdle, left shoulder girdle, and midsacral area. Animals were bled by cardiac puncture at 2 to 4 weeks after the third injection. Cortisone (Cortone:Merck and Company, Inc.) was administered in two dosages. Guinea pigs of Group I received 5 mg. per day and those of Group II received 25 mg. per day, intramuscularly. Cortisone administration was started three days before the first antigen-adjuvant injection and continued for the duration of the experiment, a total of about 4½ weeks. Complete autopsies were performed at the end of the experimental period.

Sera were allowed to remain in the refrigerator at 4° C. until hemolytic complement activity had disappeared. Analyses for antiegg albumin nitrogen were carried out in duplicate by the quantitative precipitin method of Heidelberger and Kendall[13] with the Heidelberger and MacPherson modification[14] employing the Markham apparatus[15] since only small samples of blood were obtained. Details of the procedure have been summarized.[12]

RESULTS

The antibody nitrogen content of sera at 2 and 4 weeks after the last of three weekly antigen-adjuvant injections is presented graphically in Fig. 1 and summarized in Table I. The distribution of antibody nitrogen levels in the control group is extremely varied over a wide range with no suggestion of a normal distribution curve. Therefore, the calculation of a mean titer or standard deviation has little significance. The distribution can be characterized graphically (Fig. 1) or less definitively by comparing ranges and median values. At two weeks after the last antigen-adjuvant injection, the 49 control guinea pigs had a range of 0 to 428 μg antibody nitrogen per milliliter serum (μg AbN./ml.) with a median of 123, while at 4 weeks the range of 42 control animals was substantially the same, from 1 to 330 μg AbN. per ml. with median values of 100 and 106 μg AbN./ml. The antibody levels of guinea pigs given 5 mg. cortisone daily are also widely distributed. At 2 weeks the range for 11 animals was 5 to 202; median 86 μg AbN./ml., and at 4 weeks 5 animals varied from 16 to 203 μg AbN./ml. with a median of 113. In contrast to these groups, the antibody levels of guinea pigs given 25 mg. daily are decidedly localized at the extremely low levels. At 2 weeks, 11 guinea pigs had a range of 0 to 23 μg AbN./ml., median 7; and at 4 weeks, the range of 6 animals was 0 to 24, with median values of 1 and 7 μg AbN./ml.

In the control group, granulomatous lesions were found at the inoculation sites, regional lymphoid tissue and lungs, as has been described in animals treated with similar adjuvants.[9-12, 16, 18] In addition, there was hyperplasia of lymphoid tissue generally as has been described with intensive immunization.[17] The group receiving 5 mg. cortisone was not distinguishable in these respects from the untreated control group. In the group receiving 25 mg. of cortisone, however, there was very little reaction grossly at the sites of antigen-adjuvant injection and the histologic picture was similar to that described by Kabat and associates[7] in monkeys treated with cortisone and injected with brain-adjuvant emulsion. The appearance of the tissues also resembled that present in animals which had received adjuvant emulsions lacking tubercle bacilli[12, 18] with the exception that there was somewhat more polymorphonuclear leukocytic infiltration at the local sites of injection of antigen-adjuvant emulsion in the cortisone-treated animals. The lymphoid tissue of the animals receiving 25 mg. cortisone showed a marked degree of atrophy, as has been frequently observed previously.[4, 5]

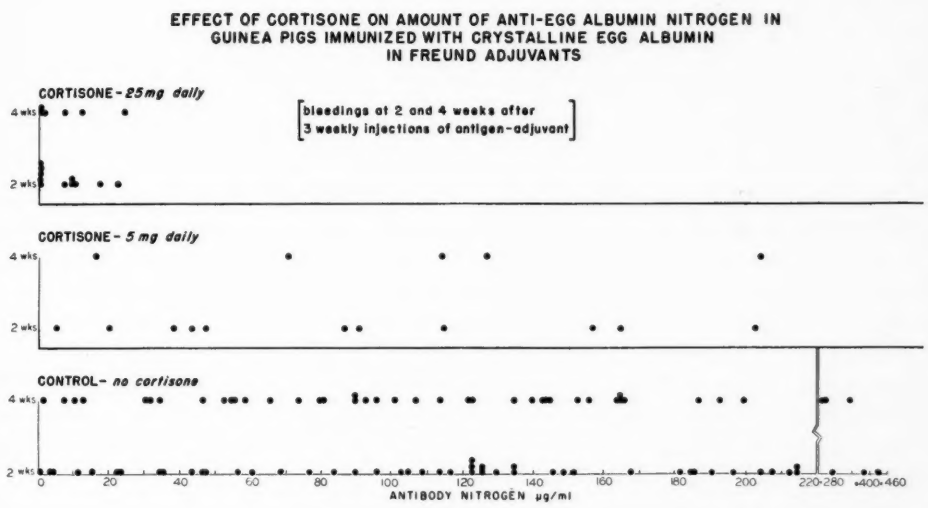

Fig. 1.

During the experimental period there was considerable mortality among the guinea pigs, particularly those receiving the larger dose of cortisone. Several animals in all groups died of unknown cause, some even before injections of antigen-adjuvant emulsion or of cortisone. It is difficult to estimate mortality rates attributable to cortisone (or to secondary infection) since repeated trauma due to injections and cardiac puncture created too many variables. In one group of guinea pigs receiving 25 mg. of cortisone daily, there were 11 deaths among 36 animals during the interval between the twelfth and twenty-fifth days of cortisone treatment, during which time intramuscular injections of antigen-adjuvant were being given but no cardiac punctures were done. In the untreated control group, 2 of 24 guinea pigs died. At another time, when controls were being immunized concurrently with the group receiving 5 mg. cortisone

daily, there were no deaths among 16 animals between the twelfth and twenty-fifth days of cortisone treatment, but during the corresponding time in the control group, 3 of 14 animals died.

Weight changes occurred in both control and cortisone-treated groups. About one-half the untreated control group lost some weight during the 4 weeks after the initial antigen-adjuvant injection. All but one of the group lost considerably more weight in the subsequent 2 weeks (between the first and second bleeding).

In the Group II animals receiving 25 mg. cortisone, 7 of 11 animals lost weight in the first 4 weeks, and all the survivors lost weight in the subsequent 2 weeks. In previous studies in rabbits,[1] it was concluded that there was no obvious correlation between weight loss or morbidity and antibody production under the conditions of the experiment. In the present study, there was no correlation between change in weight and antibody levels within the control group. Neither was there a correlation within the group treated with 25 mg. cortisone, although this was to be expected since all animals had substantially the same range of antibody. The effect of cortisone on antibody production without significant variation in body weight was adequately demonstrated by suppression of the specific anamnestic response in an experiment in which the duration of cortisone administration was not prolonged.[3]

The optimal dosage of cortisone in different animal species is a variable, apparently independent of body weight, as it also appears to be within one species—man (cf.[19, 20]). In guinea pigs, the daily dose of 25 mg. was the same as that found to be necessary to prevent wound healing and the inflammatory response in guinea pigs[20] and to protect guinea pigs from the development of adrenal lesions due to diphtheria toxin.[21] In both instances, the reactions were not inhibited by daily doses of 10 mg. or 5 mg. cortisone.[20, 22]

DISCUSSION

Cortisone inhibits antibody production when the antigen is deposited locally with Freund adjuvants as had previously been found when the antigen was administered intravenously or intracutaneously.[1,2, 8, cf. 19] Germuth and associates[8] found that smaller doses of cortisone (5 mg. per day for 18 days, then 10 mg. per day for 12 days) reduced antibody production in guinea pigs. The 5 mg. dose employed in the present study had no such effect. A complicating factor in their experiment was the introduction, on four occasions following injection of antigen with adjuvants, of a solution of egg albumin intracutaneously so that antibody levels at the time of bleeding represent the combined result of two methods of immunization. Cortisone had previously been shown to suppress antibody production when the antigen was introduced intracutaneously without adjuvants.[2] In their work, the mean titer of the control group (109 ± 17 μg AbN./ml.) is comparable to the median titers of the control group and of Group I in the present study (Table I). The mean titer of their cortisone-treated group (26 ± 7 μg AbN./ml.) is somewhat higher than the median titer of Group II animals in this study. With the amount of cortisone employed by Germuth and his co-workers,[8] a reduction in the weight of spleen and

adrenal glands was observed; microscopic examination and findings at the inoculation sites were not described.

TABLE I. EFFECT OF CORTISONE ON AMOUNT OF ANTIEGG ALBUMIN NITROGEN (ABN./ML.) IN GUINEA PIGS IMMUNIZED WITH CRYSTALLINE EGG ALBUMIN IN FREUND ADJUVANTS

	TIME AFTER THE LAST OF THREE WEEKLY INJECTIONS OF ANTIGEN-ADJUVANT					
	2 WEEKS			4 WEEKS		
TREATMENT	NO. GUINEA PIGS	μG ABN./ML. RANGE	μG ABN./ML. MEDIAN	NO. GUINEA PIGS	μG ABN./ML. RANGE	μG ABN./ML. MEDIAN
Group I						
Cortisone—5 mg.	11	5-202	86	5	16-203	113
Group II						
Cortisone—25 mg.	11	0-23	7	6	0-24	1-7
Control						
No cortisone	49	0-428	123	42	1-330	100-106

Intensive immunization is followed by hyperplastic changes in lymphoid tissue.[17] Large doses of cortisone result in striking suppression of lymphoid tissue.[4, 5] Antigen deposited locally with Freund adjuvants results in local granuloma formation and granulomatous changes in the regional lymph nodes, lungs, spleen, and elsewhere,[9-11] together with a peculiar "halo" of fibrous tissue around the malphigian corpuscles.[12] With the larger dosage of cortisone in the present study, diminution of antibody formation was strikingly correlated with absence of the granulomatous response. The low antibody levels to egg albumin and the absence of a granulomatous response also occurred when tubercle bacilli were omitted from the antigen-adjuvant emulsion.[12] In these respects, therefore, it appears that cortisone administration inhibits the inflammatory reaction due to tubercle bacilli. In both experiments, it appears that the degree of granulomatous reaction has an intimate relationship to the development of antibody. Furthermore, the development of experimental encephalomyelitis is inhibited by either omission of tubercle bacilli from the brain-adjuvant emulsion[18, 23, 24] or by the administration of corticotropin[6] or cortisone[7]; omission of the tubercle bacilli[18, 23, 24] or treatment with cortisone[7] strikingly reduced granuloma formation at the inoculation sites. These findings furnish additional evidence compatible with the hypothesis that experimental disseminated encephalomyelitis involves an immunologic mechanism.

SUMMARY

The administration of large dosages of cortisone to guinea pigs receiving crystalline egg albumin with the Freund adjuvant resulted in marked reduction of antibody levels and in marked diminution of granuloma formation at the site of the antigen-adjuvant injection. The relationship of granuloma formation to antibody production is discussed in the light of these studies together with previous work on the omission of tubercle bacilli and on experimental encephalomyelitis.

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MAINTENANCE CORTISONE† IN INTRACTABLE ASTHMA

Preliminary Observations of Undesirable Cortisone Effects

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With the technical assistance of Priscilla Gordon and Mary Gilchrist, B.S.

INTRODUCTION

PATIENTS with constant, intractable, perennial asthma of unknown etiology have always presented a perplexing therapeutic problem. In this group a few patients become resistant to the usual forms of therapy, and these unfortunate individuals gradually develop into economic, physical, and social invalids. Their plight and the concern of their physicians are so marked that almost every new agent of promise is soon tried.

When cortisone promised such hope in rheumatoid arthritis, an equally puzzling disease, this potent hormone was soon used in asthma. Within a brief period of time, cortisone proved to be effective in asthma of all types, but unfortunately the symptoms usually returned once the cortisone was stopped. Its proper position, therefore, fell into the category of symptomatic therapy. It is now generally agreed that the only role for cortisone in the treatment of allergic asthma is the relief of symptoms until allergic measures can be brought to bear. Still unproved is the feasibility of prolonged cortisone therapy for patients with severe asthma of undetermined etiology.

In April, 1951, six such patients were placed on cortisone in dosages large enough to suppress symptoms and were maintained on daily cortisone. From time to time, other patients have been added to the group, but the group is still a small one. Burrage and associates[1, 2] have reported earlier data on these patients. The purpose of this paper is to bring these observations up to date.

METHODS

Each patient had constant, intractable perennial asthma of undetermined origin and was known to the staff for at least one year prior to this study. The usual forms of therapy had failed to permit these patients to assume any useful place in either the social or economic spheres of life. As a further check each was hospitalized and given intense, routine emergency treatment, including good

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†Cortisone used in this study was supplied in part through the courtesy of Merck and Co., Inc.

nursing care, proper fluids, epinephrine, and aminophylline for at least forty-eight hours before cortisone was used. If the patient improved on routine therapy, such therapy was continued. Only after the patient failed to improve, was he considered for prolonged cortisone therapy.

Every patient in this group was studied with care before cortisone therapy was instituted. A complete history and thorough physical examination were done. Special search was made for such possible contraindications as tuberculosis, gastrointestinal bleeding, diabetes, thromboembolic phenomena, and neuropsychiatric disorders. Roentgenogram of the chest, routine urinalysis, complete blood count, fasting blood sugar, electrocardiogram, vital capacity, circulating eosinophils, serum sodium, serum potassium, serum chloride, blood nonprotein nitrogen, and usually glucose tolerance tests were obtained. Each patient was placed on a low-sodium diet of 1,200 calories. The caloric intake was adjusted later to each patient's needs. In retrospect it appears that several calcium determinations on 24-hour urine collections, serum calcium, serum inorganic phosphorus, serum alkaline phosphatase, and roentgenograms of the anteroposterior and lateral views of the dorsal and lumbar vertebrae should have been done prior to therapy.

Each patient on cortisone has been followed carefully. This has included a complete physical examination at least twice a month as well as frequent laboratory studies. The patients report immediately the slightest variation from normal.

DOSAGE OF CORTISONE

The dosage of cortisone has varied from patient to patient, but a certain general pattern has been followed. Sufficient cortisone was given initially to suppress the symptoms of asthma completely. The following schedule has been used in adults: 75 mg. every 6 hours for 24 hours and then 50 mg. every 6 hours until complete suppression of symptoms occurs. Once the symptoms are suppressed, the daily maintenance dosage is found by "trial and error." The daily dose is decreased once every week. The point is eventually reached at which symptoms begin to reappear, and then the daily dose is established at 12.5 to 25 mg. above this level. To date, all in this series have required at least one daily dose of cortisone to remain symptom-free. All efforts to establish adequate maintenance by giving one to three doses a week have led to failure. After the maintenance level has been determined, it fluctuates very little. Upper respiratory infections and periods of stress do tend to increase the requirements temporarily. The following case report will illustrate many of these points.

CASE REPORT

CASE 1.—P. B., white man, married, 72 years of age. His first attack of asthma occurred at age 59 in May, 1941; his asthma was not seasonal and failed to clear on changing environment. At first the one or two daily attacks responded readily to subcutaneous injections of epinephrine, 1 to 1000. In 1942, at age 60, his attacks became more severe and frequent. His sleep was interrupted, and he often sat up most of the night as ephedrine, epinephrine, and iodides no longer possessed their previous effectiveness. There was little change until 1949, when at age 67 severe asthma forced him to stop work. Hospital visits

became more frequent, and each visit became more protracted. During one attack he lost consciousness and was placed on the danger list. Following this episode his doctor persuaded him to move to Tucson, Arizona, where he remained eight months. There, partial control was attained by daily intravenous aminophylline and frequent use of epinephrine. He became discouraged and returned home to Cape Cod where he wheezed and could not work in spite of subcutaneous epinephrine every 3 hours and intravenous aminophylline every 12 hours. He entered the Massachusetts General Hospital on April 30, 1951. His past medical history was not contributory, but it was noted that both his wife and son had asthma.

Physical examination revealed a tense, white man, propped up in bed, appearing older than his stated age, and wheezing audibly. The following data were obtained: weight, 153 pounds; height, 66¾ inches; blood pressure, 160/90 mm. Hg; temperature, 98.6° F.; respiratory rate, 24 per minute; and pulse rate, 60 per min. Polypoid degeneration of the nasal mucosa was found. The anteroposterior diameter of the chest was increased; the excursion of the diaphragm was limited; the chest was hyperresonant; and all lung fields were full of inspiratory and expiratory musical râles.

Laboratory studies were done before cortisone therapy. Venous pressure was 110 mm. of saline, and vital capacity (3 seconds) was 2.2 liters. Roentgenograms included: teeth, slight periodontoclasia; sinuses, slight thickening of mucous membranes of both antra; chest, emphysema and slight rounding of heart as well as tortuosity of aorta; thoracic vertebrae, slight osteoporosis. An electrocardiogram showed bradycardia. His urinalysis and routine complete blood count were within normal limits, but his circulating eosinophils numbered 360 per mm.^{3} Multiple skin tests by both the scratch and intracutaneous methods were all negative.

At 6 P.M. May 3, 1951, he was given 75 mg. of cortisone intramuscularly and remained on this dose every 6 hours for twenty-four hours. Fifty milligrams every 6 hours were then ordered. After four such injections, his improvement was so marked that the dose was lowered to 37.5 mg. every 6 hours. On May 6 at 6 P.M. the dose was further lowered to 25 mg. every 6 hours. His asthma cleared May 9. On May 10, he was discharged on 25 mg. intramuscularly every 8 hours. All went well so, on June 24, his dose was decreased to 17.5 mg. every 8 hours. On July 29, 1951, it was lowered to 12.5 mg. at similar intervals, a total daily dose of 37.5 mg. Wheezing recurred at this level, but no change was made until Sept. 22 when his symptoms became so persistent that his dose was raised to 50 mg. daily, and on this the asthma cleared. On Oct. 21, 1951, he was placed on 37.5 mg. daily, and again his asthma returned. When the dose was raised to 50 mg., he did well until Feb. 9, 1952, when recurrent asthma necessitated a further rise to 75 mg. daily. In April, 1953, he was switched to a similar amount of oral cortisone. Asthma soon returned. One hundred milligrams of oral cortisone were then prescribed in two divided doses, and he has remained symptom-free on this dose for the past 8 months.

On Jan. 8, 1954, he appeared in excellent health. He weighed 162 pounds and measured 66¾ inches in height. The nasal mucosa no longer showed polypoid degeneration. Roentgenograms of the thoracic and lumbar vertebrae showed no increase in osteoporosis. Urine calcium excretion was 150 mg./24 hours. No significant changes have ever been noted in his blood pressure, serum potassium, serum sodium, serum chloride, blood nonprotein nitrogen, fasting blood glucose, or glucose tolerance curve. His three-second vital capacity was 3 liters. Since leaving the hospital in May, 1951, he has worked six days a week. He does show rounding of facial contours and has a high facial color.

All members of this small group have been cleared of asthma and have been maintained symptom-free. Fig. 1 shows the amount of cortisone necessary to clear each patient as well as the length of time required. It will be noted that most patients required approximately one gram given over a period of 5 to 7 days. Several patients required less, some more. Fig. 2 shows the range

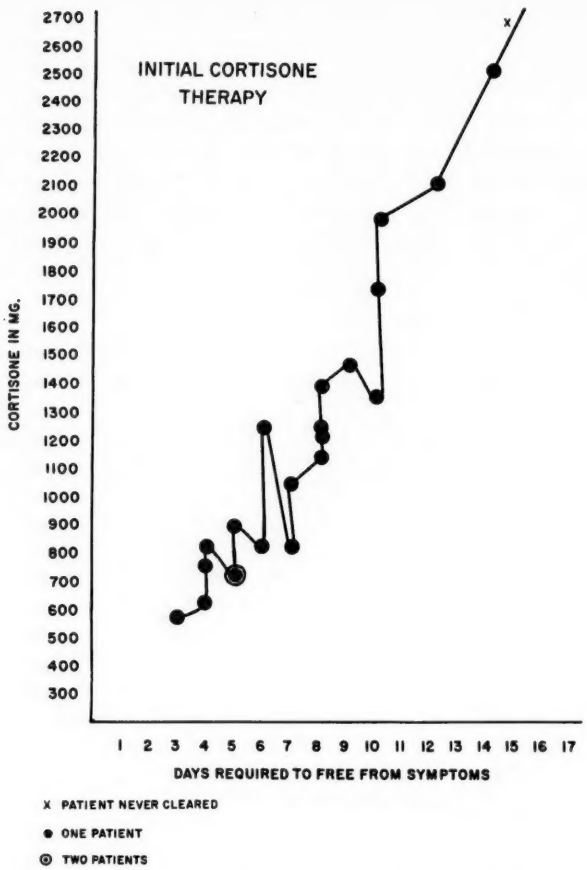

Fig. 1.—The axis of ordinates shows the total amounts of cortisone in milligrams needed to clear the patients of asthma. The axis of abscissas illustrates the number of days before complete cessation of asthma. Each point on the graph represents one patient and has as its coordinates the total amount of cortisone in milligrams and the number of days needed to free that patient of asthma.

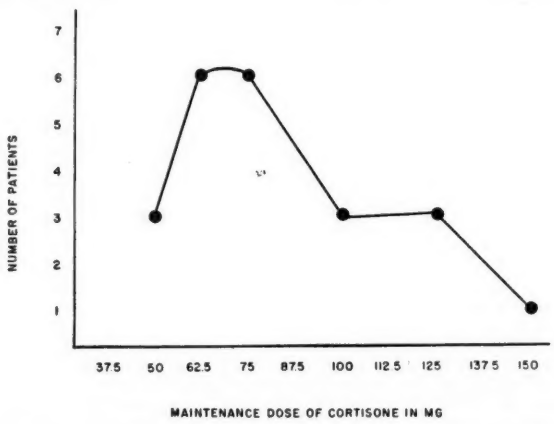

Fig. 2.—The axis of ordinates shows the number of patients. The axis of abscissas lists the total daily dose of cortisone in milligrams. Each point on the graph points out the number of patients maintained symptom-free from asthma on a particular daily dose of cortisone.

of daily maintenance levels of the patients. In general the average maintenance dose appears to be 75 to 100 mg. daily. Individual variations, however, require careful consideration of each patient.

COMPLICATIONS

Several problems arose which were probably unrelated to the pharmacologic action of cortisone. All of the patients have had minor respiratory tract infections, and two patients required brief hospitalizations for tracheobronchitis. During intramuscular cortisone therapy, three patients developed abscesses at sites of injection; in fact, one of these had a second abscess at a later date. In each instance, sterile precautions had been ignored, and the abscesses healed on surgical drainage and antibiotic therapy. One patient developed a cheilosis which cleared readily on vitamin therapy.

UNDESIRABLE CORTISONE EFFECTS

Since the daily dose of cortisone required for suppression of this type of asthma would appear to be two to three times the normal rate of secretion of cortisone-like hormone as estimated by Norymberski and associates,[3] it may be

TABLE I. POSSIBLE MANIFESTATIONS OF CHRONIC CORTISONE THERAPY

MILD	SEVERE
1. Obesity	Gastrointestinal bleeding
2. Edema	Vascular disease
3. Acne	Psychosis
4. Hirsutism	Osteoporosis
5. Decreased carbohydrate tolerance	Growth arrest
6. Symptomatic metabolic alkalosis	Edema
7. Menstrual changes	Hypertension
8. Symptomatic potassium deficiency	Acceleration of infection

TABLE II. WEIGHT CHART

NAME	SEX	WEIGHT BEFORE CORTISONE	IDEAL WEIGHT	WEIGHT DECEMBER, 1953
B. B.	F	116.6	114-122	125.5
B. C.	F	128.0	116-125	134.0
H. P.	M	177.0	174-190	183.0
R. W.	M	168.5	157-168	186.5
M. W.	F	110.0	110-118	118.0
T. G.	M	163.0	157-168	166.0
J. P.	F	116.0	124-132	131.0
A. B.	F	123.5	110-118	136.5
M. A.	F	94.5	110-118	114.0
D. P.	F	141.5	141-151	142.5
E. W.	M	175.0	149-160	177.5
E. K.	F	153.0	148-158	164.5
M. M.	F	114.0	127-135	140.0
F. S.	F	122.0	113-121	131.0
P. B.	M	153.0	141-151	162.0
W. G.	M	162.0	134-144	150.0
M. D.	F	85.0	120-128	139.0
C. R.	M	170.0	161-175	179.0
N. Z.	F	125.0	133-145	146.5
E. M.	F	127.0	120-128	167.0
D. M.	M	168.0	141-153	187.0
A. W.	F	147.0	131-142	155.0

anticipated that such hyperadrenocorticism will in time reproduce at least part of the clinical syndrome of spontaneous hyperadrenocorticism or Cushing's syndrome. Table I lists the possible mild and severe manifestations of long-term cortisone therapy. A survey of the twenty-three patients shows that most have developed one or more of the mild manifestations. Four female patients have an increase in the amount of facial hair. Two male patients have developed acne. Menstrual function has remained essentially normal in premenopausal women. Decreased carbohydrate tolerance, edema, and symptomatic potassium deficiency have been absent to date. All patients show facial rubor and rounding of facial contours. Table II illustrates the weights of these patients before cortisone therapy, the calculated ideal weights for each patient, and the weight of each as of December, 1953. All of the patients except one have gained weight, and seventeen are over their ideal weight. Furthermore, all except four have an abnormal distribution of weight with increased fat in the abdominal, facial, supraclavicular and lower cervical areas.

The serious manifestations of Cushing's syndrome include osteoporosis and growth inhibition, hypertension, vascular disease, psychosis, acceleration of infection, and debility. Among the 43 unpublished cases of Cushing's syndrome studied by the Fuller Albright group at the Massachusetts General Hospital, osteoporosis was one of the most common serious symptoms, being present in 39 patients. Such osteoporosis appeared as soon as several months after recognition of the pathologic state by the patient and frequently produced back pain, occasionally with radicular distribution. The serum calcium, phosphorus, and alkaline phosphatase were normal, but the urine calcium excretion was increased in most of the patients tested. Roentgenograms demonstrated generalized increased radiolucency of the bones which was most marked in the spine and ribs. Also characteristic were increased or decreased trabecular markings, dense end plates as compared with the central portion of the vertebrae, widening of the intervertebral spaces, and finally collapse of vertebrae, most commonly in the lumbar and thoracic areas.

The first patient (W. G.) in the group on maintenance cortisone to develop severe osteoporosis was a 60-year-old, white man who had had severe asthma for twenty-five years. In late 1951, he received cortisone therapy for three months and was placed on maintenance cortisone in August, 1952. The upper row of tracings of thoracic spine roentgenograms in Fig. 3 illustrates the progressive collapse of many vertebrae with increasing kyphosis in the past two years. His height, originally 69 inches, had decreased to 68¼ inches at age 51, to 67 inches in May, 1952, at age 59, and to 66 inches in recent months. During the last eight months, he has been severely handicapped by severe back pain, and since July, 1953, has demonstrated loss of muscle mass and sensation in his right hand, presumably due to compression of the cervical nerve roots. Most instructive has been his marked hypercalcuria in the range of 300 mg. daily while on cortisone. Note that his urine calcium fell to 50 mg. when cortisone was stopped, only to rise to over 250 mg. per day on return to cortisone therapy.

Since Bartter and associates[4] demonstrated that testosterone can modify some of the effects of corticotropin, testosterone was given to W. G. after he

developed symptomatic osteoporosis. Early evidence indicates a reduction in his urine calcium while on testosterone and some relief of back pain. The effect of testosterone and other agents on the undesirable effects of prolonged cortisone therapy is under present investigation.

The negative calcium balance produced by cortisone reflects loss of bone mass inasmuch as the bones and teeth contain 99 per cent of body calcium. Such a negative balance is due to an increase in urine calcium excretion. Indeed, urine calcium excretion is the most convenient clinical index of bone metabolism and may be of special value in following patients on cortisone therapy. Fig. 4 shows the 24-hour urine calcium excretion of the patients on maintenance cortisone therapy. Calcium excretion in excess of 150 mg. daily may indicate loss of bone mass, and it is probable that those patients with high urine calcium excretions will be the first to develop symptomatic osteoporosis.

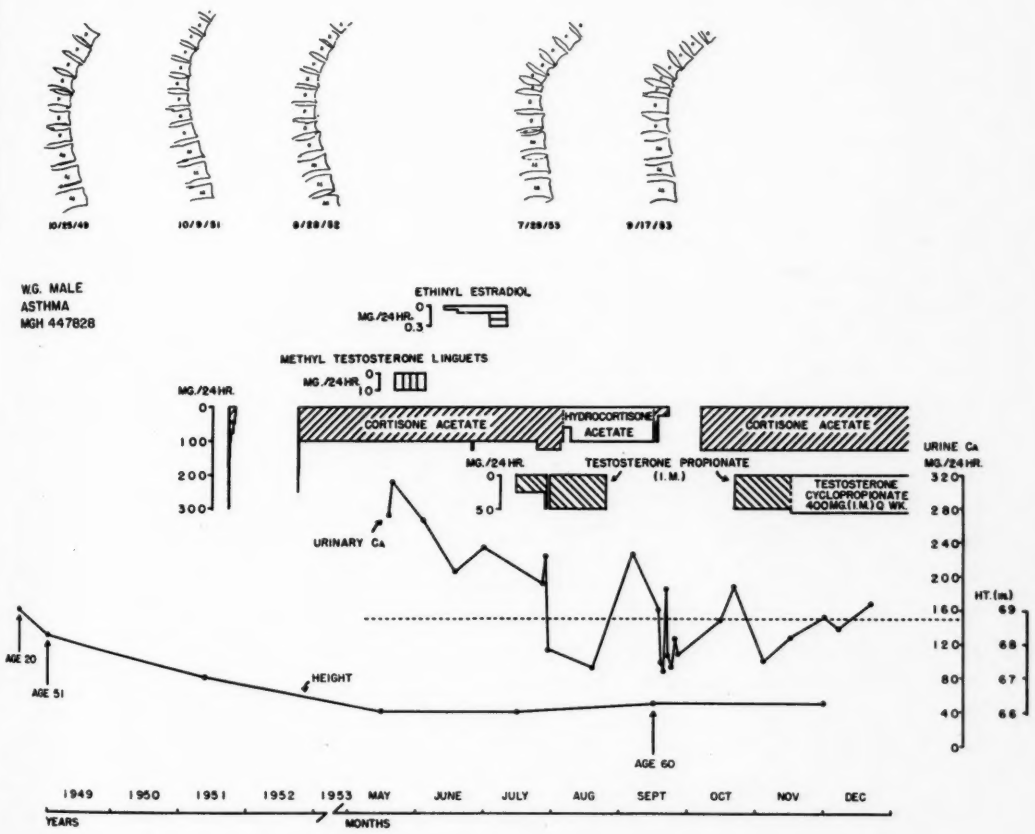

Fig. 3.—A composite graph of findings on W. G. The upper tracings are those taken from roentgenograms of thoracic vertebrae. All five show kyphosis. The first three were made before cortisone therapy, and one vertebra has already fractured. The last two were made during cortisone therapy and show rapid progression of osteoporosis with several fractures. Cortisone dosage and testosterone therapy are correlated with the 24-hour urinary calcium excretion. Note that cortisone tended to increase calcium excretion and that testosterone tended to depress calcium excretion. The height line shows a decrease in height through the years before cortisone with some drop since cortisone therapy.

CONCLUSIONS AND SUMMARY

The studies of the last three years demonstrate that most patients with severe, intractable, perennial asthma can be relieved of their symptoms and maintained symptom-free with cortisone. Enough cortisone must be given initially to relieve the patient completely of symptoms. The daily maintenance

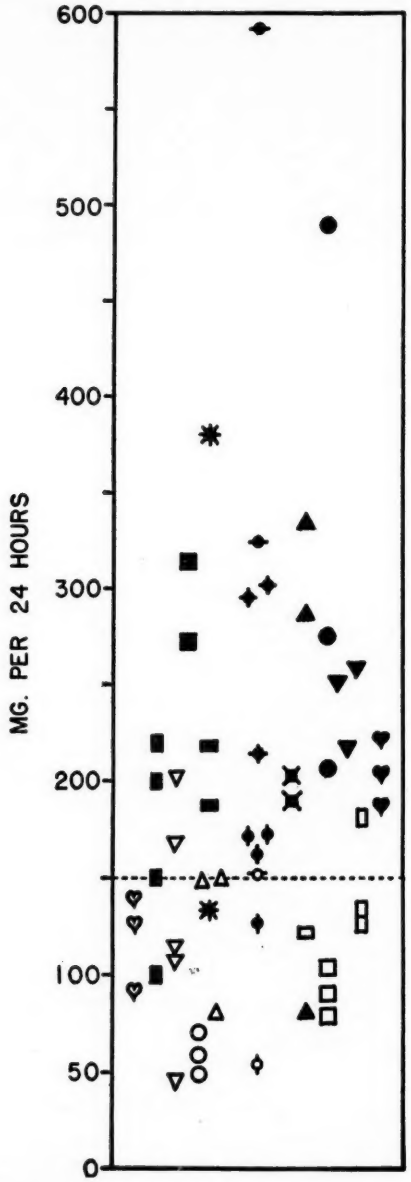

Fig. 4.—Urine calcium excretion of 23 patients with asthma on prolonged cortisone therapy. Each symbol represents one patient, and is placed in regard to the number of milligrams of calcium excreted in the urine in 24 hours. For instance, the open circle illustrates that one patient excreted 50 mg. calcium in the urine in one 24-hour period, 70 mg. in another similar period, and 75 mg. in another 24-hour period.

dose should be as small as possible and can be determined only by repeated "trial and error." Even such maintenance cortisone represents hyperadrenocorticism and appears capable of producing the more serious manifestations of Cushing's syndrome including osteoporosis. Determinations of urine calcium excretion and periodic spine roentegenograms may prove to be useful guides in predicting which patients on cortisone may develop clincal osteoporosis. Patients on maintenance cortisone must be watched closely.

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RELEASE OF HISTAMINE IN THE BLOOD OF RAGWEED-SENSITIVE INDIVIDUALS

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A FEW investigators have reported that the exposure of sensitized blood cells to a specific antigen results in the liberation of histamine into the plasma. This reaction may be elicited by adding homologous antigen either to whole blood of actively sensitized animals[1-3] or to whole blood obtained from patients with allergic symptoms known to be due to an antigen such as that present in ragweed pollen.[4]

Katz,[5] in summarizing his studies of histamine release, indicated that the reaction was dependent upon exposure of sensitized blood cells to the specific antigen involved. The reaction took place readily when the blood cells were freed of plasma and suspended in saline solution before being exposed to the antigen, indicating that the reaction was strictly a cellular one, not mediated by any substance present in the plasma.

Previous investigators of the histamine release phenomenon have employed the biologic method of histamine assay. Graham and associates[6] have developed a chemical method of histamine assay which is dependent upon the formation of a colored conjugation compound of dinitrofluorobenzene and histamine, the concentration of which can then be determined spectrophotometrically. This technique was employed in our studies. By the method, amounts of histamine added to plasma are recovered within a range of plus or minus 10 per cent.

METHODS

Venous blood was obtained from proved ragweed-sensitive patients (hay fever, asthma) who were free of symptoms at the time and who, at the time of obtaining the first blood sample, had not been exposed to ragweed pollen for five months. All patients except one were receiving periodic hyposensitizing injections of ragweed extract, several of them having received injections as recently as one week prior to the time blood was obtained. In five, it was possible to obtain a second blood sample during the fourth week of the ragweed pollen season, when all five were having mild symptoms of pollinosis. All blood specimens were collected in syringes wet with a solution containing 10 mg. of sodium heparin per milliliter. Approximately 50 ml. of blood were obtained from each individual and divided into two equal parts. To one portion was added 0.25 ml.

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of ragweed extract containing 2,500 protein nitrogen units of ragweed antigen. The other remained untreated. Both portions were then incubated in a water bath at 37° C. for 20 minutes. After these procedures on the whole blood of four individuals, the blood was assayed for histamine. A blank containing 0.25 ml. of ragweed extract, 1 ml. of 4 M. sodium acetate, and 5 ml. of 5 per cent trichloroacetic acid, the protein precipitant used in this method, was run with each pair of determinations. Results are listed in Table I.

TABLE I. HISTAMINE DETERMINATIONS OF WHOLE BLOOD PLUS ANTIGEN

PATIENT	BLOOD HISTAMINE	BLOOD (PLUS RAGWEED) HISTAMINE
F. T.	130	136
B. G.	129	133
J. M.	130	112
W. H.	113	128

Figures represent micrograms of histamine per liter of whole blood.

Treated in the same manner as those appearing in Table I, the remaining ten pairs of blood samples were centrifuged, the plasma removed, and the histamine content of the plasma determined. Results appear in Table II.

TABLE II. HISTAMINE DETERMINATIONS OF THE PLASMA FROM WHOLE BLOOD PLUS ANTIGEN

PATIENT	PLASMA FROM BLOOD WITHOUT RAGWEED ANTIGEN	PLASMA FROM BLOOD PLUS RAGWEED ANTIGEN
M. J.	4	10
G. C.	3	34
L. H.	4	63
C. St.	4	35
R. D.	5	131
R. C.	6	44
D. P.	3	23
L. M.	3	19
J. J.	2	29
C. Sc.	3	9

Figures represent micrograms of histamine per liter of plasma.

TABLE III. CONTROL DETERMINATIONS ON THE BLOOD PLASMA OF FIVE NONSENSITIVE INDIVIDUALS

PATIENT	PLASMA FROM BLOOD WITHOUT RAGWEED ANTIGEN	PLASMA FROM BLOOD PLUS RAGWEED ANTIGEN
J. N.	5	7
A. B.	24	17
R. E.	3	6
R. D.	4	7
H. I.	7	8

Figures represent micrograms of histamine per liter of plasma.

During the ragweed season blood was obtained from five of the ragweed-sensitive individuals whose blood histamine release had been determined a few months previously. All individuals had been under regular hyposensitizing treatment in the interval between the two determinations as well as for a variable period prior to that. All had shown a good response to therapy.

In Table IV, some figures shown in Table II are again presented for comparison.

TABLE IV. COMPARATIVE DETERMINATIONS OF PLASMA HISTAMINE VALUES BEFORE AND DURING THE POLLEN SEASON

PATIENT	PLASMA FROM BLOOD WITHOUT RAGWEED ANTIGEN		PLASMA FROM BLOOD PLUS RAGWEED ANTIGEN	
	March	September	March	September
C. S.	4	4	35	54
R. C.	7	7	44	58
D. P.	3	7	23	62
L. M.	3	4	19	68
J. J.	2	13	29	75

Figures represent micrograms of histamine per liter of plasma.

DISCUSSION

When the results of histamine determinations of the whole blood of ragweed-sensitive individuals are examined (Table I), it is apparent that there is no significant variation in the "blood" and "blood plus ragweed" figures. Total blood histamine is largely in ratio to the total leukocyte count. One does not increase total blood histamine appreciably by adding antigen to a sensitive individual's blood. In these four pairs of determinations the largest increase, of 15 μg per liter, is but 13 per cent. The lack of significance of this increment is emphasized by the large and constant increase in the value of plasma histamine found when blood of sensitive individuals is incubated with antigen. The percentile increase varies from 180 to 2,520 per cent, with an average increase of over 900 per cent. By coincidence, 900 per cent is also the figure given by Katz and Cohen[4] as the greatest plasma histamine increase obtained in two of ten sets of determinations they selected as examples. The amount of histamine released in these two instances was 27 and 45 μg per liter. Table II and Table IV show that the largest amount released in our experiments was 126 μg per liter, and in seven instances was over 50 μg per liter. Our values were, therefore, generally higher, for reasons not determined but probably inherent in the methods used. Since the blood of nonallergic individuals showed no significant histamine release (Table III) when treated with antigen, compared with the blood of those who had hay fever, it is apparent that the release phenomenon depends upon allergic sensitivity.

It has been reported that erythrocytes as well as white cells participate in the histamine release reaction, but to what extent each cell type contributes its histamine is not known. One might assume that cellular types high in histamine contribute the most. From this viewpoint, figures arrived at in the histamine assay of red and various white blood cell elements are important. With the dinitrofluorobenzene technique, the approximate contribution of various blood elements to the total blood histamine has been determined as follows[7]:

Erythrocytes	20 per cent
Neutrophils	50 per cent
Basophils	15 per cent
Eosinophils	1 per cent
Platelets	3 per cent
Lymphocytes	1 per cent
Plasma	4 per cent

With the chemical method, the average total blood histamine value of 122 μg per liter is high when compared with values obtained by biologic assay.[8] This may be due to the fact that chromogens other than histamine are detected by the dinitrofluorobenzene method. The color-producing substance or substances present in human erythrocytes are not altered by exposure to histaminase and are, therefore, not histamine. If one determines only the chromogenic fraction of human whole blood which is acted upon by histaminase, an average normal value of 82 μg per liter is found.[9] This figure more nearly approaches values obtained by the biologic method.

In our determinations, occasionally more than 60 per cent of the whole blood histamine, calculating from the average normal value of 122 μg per liter, has been released into the plasma after addition of antigen. The neutrophils, containing 50 per cent of the total blood histamine, must have contributed, and probably the basophils and other white cell elements as well. The blood samples obtained during the fourth week of the ragweed season, from patients with symptoms of ragweed pollinosis, showed a greater histamine release (300 per cent increase) when compared with nonseasonal values. Five of the seven histamine release values exceeding 50 μg per liter were in the coseasonal group. The number of determinations made is so far too small to permit any valid conclusions. One may speculate that since plasma histamine values remain about the same in and out of the ragweed season, the increased amount of histamine released during the ragweed season is quickly removed from the plasma, and either the blood cells, for reasons unknown, give up their histamine more readily during the season, or the number of leukocytes in the blood is greater at this time. During the ragweed season, total white blood cell counts were done on the samples obtained and these were within normal limits. Unfortunately, total white counts were not performed on the samples obtained previously. Regardless of the fundamental cause of greater histamine release during the ragweed season, it is possible that the increased liberation of histamine in coseasonal samples may offer an explanation of the higher incidence of constitutional reactions during the ragweed season when patients are being hyposensitized on conventional perennial treatment schedules.

CONCLUSIONS

1. An appreciable release of histamine into the blood plasma was detected when whole blood of a clinically sensitive individual was incubated with ragweed antigen.

2. No such increase appeared in unseparated whole blood, similarly treated.

3. The discrepancy is attributed to a transfer to the plasma of histamine contained in blood cells.

4. Greater release of histamine occurred in blood samples taken during the ragweed season than in those taken preseasonally.

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COMPLEMENT FIXATION STUDIES IN RAGWEED ALLERGY

I. Quantitative Complement Fixation Studies With Ragweed-Pollen Extract and Homologous Rabbit Antisera

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INTRODUCTION

ALTHOUGH our knowledge of the basic mechanisms operating in various allergic phenomena is still incomplete, a causal relationship of antibodies with most allergic conditions has been established. The chief tool employed in the demonstration of such antibodies has been an in-vivo procedure developed by Prausnitz and Küstner,[1] which has come to be known as the passive transfer method.

By using the passive transfer method two types of antibodies have been recognized in ragweed allergy: skin-sensitizing (reaginic) and blocking (inhibiting, neutralizing) antibodies. The existence of skin-sensitizing antibodies in ragweed allergy was first noted by de Besche[2] and confirmed by Coca and Grove.[3] The presence of another type of antibody, considered to be distinct from skin-sensitizing antibody, was noted by Cooke and associates[4] and designated as blocking antibody. It was observed that blocking antibody, unlike sensitizing antibody, was developed in allergic or nonallergic individuals as a result of injection treatments with ragweed antigens. The existence of blocking antibody was confirmed by Harley,[5] Langner and Kern,[6] Loveless,[7] and others.[8-18]

Attempts have been made in the past to determine the presence of the two types of antibodies in ragweed allergy by use of various in-vitro methods. The absence of precipitins in ragweed sensitive sera was noted by Coca and Grove.[3] Cohen and Weller,[19] using a collodion particle technique, reported the presence of precipitating antibodies in the sera of ragweed-treated, ragweed-sensitive patients but not in untreated ragweed-sensitive individuals. However, negative findings were obtained by Swineford and Houlihan[20] and by Cavelti[21] with both treated and untreated ragweed-sensitive donors.

The determination of blocking antibody through its inhibiting effect on the precipitin reaction of ragweed antigen and rabbit antibody was first made by Hampton and associates.[22] The method was further refined by Johnson and associates,[23] but in a later application of this method by Alexander and

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associates,[24] a lack of correlation between the blocking antibody titer and the degree of clinical protection was observed. Through consideration of the possibility that the volume of precipitates measured by the procedure described[23] might not be perfectly correlated with the absolute amounts of precipitates, Bukantz and associates[25] adapted the quantitative precipitin technique of Heidelberger and MacPherson[26] to the measurement of the precipitates obtained by the reaction of whole ragweed extracts and antiragweed rabbit serum. Included in their publication were some preliminary observations on the behavior of blocking antibody in the system. It was noted that a given amount of serum containing blocking antibody appeared to neutralize a fixed amount of antigen independently of the antigen-antibody ratio of the precipitating mixture. Normal serum appeared to possess some inhibiting activity, but this was much less than the effect noted for a serum pool prepared from a treated ragweed donor.

Complement fixation has likewise been employed in the study of ragweed antibodies. Thus Albus[27-29] studied the antibodies in hay fever by the use of the Jaffé method,[30] which utilizes native human complement and antisheep hemolysin, and concluded that the complement fixation reaction was a more useful guide than skin tests in both the diagnosis and treatment of hay fever. Following specific desensitization he regularly observed a decrease in the complement-fixing titer which he interpreted as evidence of the neutralization of reagin by the injected antigen. Hensel and Sheldon[31] likewise studied the sera of hay fever patients by the Jaffé method, using extracts of short ragweed, June grass, and orchard grass pollens as antigens. Positive reactions were noted in most untreated patients who were sensitive by the scratch test, but there was no correlation between the complement-fixing titer and the degree of skin reaction. Cavelti,[21] using a quantitative complement fixation test patterned after that of Mayer and associates,[32] found that a high proportion of cases who were sensitive by skin tests had complement-fixing antibodies to epidermal antigens. However, a lower incidence of reactions was observed with pollen antigens and, in addition, these reactions were all of very low intensity. Cavelti considered that the weakness of these reactions might have been due to the lesser potency of the ragweed antigens used. There was no indication that sensitizing and blocking antibodies could be distinguished by the complement fixation reaction. More recently, Follensby[33] studied the complement-fixing properties of sensitizing and neutralizing antibodies, using ragweed and timothy extracts as antigens. No evidence of direct fixation of complement was noted when either native (human) or guinea pig complement was used. Inhibition of fixation of complement by antiragweed and antitimothy rabbit sera in the presence of allergic sera was observed, but in view of the similar behavior of normal sera the specificity of the reactions with the allergic sera was questionable.

Several other in vitro procedures based on antigen-antibody interaction have been described in connection with ragweed allergy. Thus Squier and Lee[34] reported the in vitro lysis of leukocytes resulting from the addition of

short ragweed antigen to heparinized whole blood of patients sensitive clinically and by skin tests to this antigen. Franklin and Lowell[35] failed to confirm these findings. More recently, Bernard and associates[36] used the method of Squier and Lee to study certain aspects of hypersensitivity and anaphylaxis in dogs, using dog hemoglobin as antigen, but found no significant differences in the total leukocyte counts among sensitized and nonsensitized animals.

Spain and associates[37] reported that histamine was released by the serum of untreated ragweed-sensitive subjects after incubation with ragweed antigen and normal rabbit blood. The normal rabbit blood was used to supply leukocytes, which contain bound histamine. It was found that neither normal human serum nor the serum of allergic persons after specific treatment released histamine from such mixtures. It was concluded that the release of histamine was governed by antigen-antibody interaction. In a later article,[38] it was shown that oleic acid inhibited the in vitro release of histamine, and this was offered as evidence for enzymatic action being involved in the reaction described.

From the literature, it therefore appears that the in vitro tests which have been applied to the study of ragweed allergy have either had a limited investigation or have not met the requirements of a satisfactory procedure. Because of the difficulties inherent in the performance of skin tests as well as in their interpretation, there appears to be a need for a simple and reliable in vitro method for determining the presence of the antibodies concerned in allergic disease. The development of such a procedure would have application to the diagnosis, treatment, and study of the immunology of allergic disease.

Attention was directed to the use of the complement fixation reaction for the determination of allergic antibodies by recent observations of Rice,[39-43] who noted that certain immune animal sera were incapable of directly fixing complement in the presence of the appropriate antigen but nevertheless inhibited the complement-fixing properties of serum from another animal species. Rice termed this reaction the "indirect complement fixation test." Prior to Rice's work, other instances had been noted[44-47] in which antibody precipitated with its antigen, failed to fix complement, and inhibited the fixation of complement by a complement-fixing antiserum. These observations, however, had never been applied to the study of the incidence of specific diseases. The principle elaborated by Rice has since been employed by other investigators in the study of certain viral diseases.[48-52]

Since blocking antibody found in ragweed allergy showed the property of inhibiting the precipitation of ragweed antigen-rabbit antibody,[22-25] it seemed likely that such antibody might likewise inhibit the fixation of complement which occurs when ragweed antigen and rabbit antibody react. Preliminary studies by the author suggested that such inhibitory properties were possessed by sera containing blocking antibody by skin tests.

The purpose of the work was to develop, if possible, a complement fixation inhibition test which could be used for the study of blocking antibodies in rag-

weed allergy. It was necessary first to study the complement fixation reaction of ragweed antigens and specific immune rabbit serum in order to determine optimal conditions for the detection of blocking antibody. Finally, a comparative study was made of the complement fixation inhibition test and the passive transfer test for blocking antibody to determine the relationship of these two tests. The studies reported herein have been divided accordingly into companion papers:

1. Quantitative complement fixation studies with ragweed pollen extracts and homologous rabbit antisera.

2. Determination of antibody in human sera to ragweed antigen by means of a complement fixation inhibition test; the relationship of antibody so determined to the passive transfer test for blocking antibody.

MATERIALS AND METHODS

Antigens.—

A. Preparation and standardization: Whole extract antigens were prepared using Coca's fluid (alkaline, hypotonic saline), with or without 0.4 per cent phenol. The nonphenolized extracting fluid was employed to prepare the antigen used in immunizing rabbits. The procedures followed for the preparation of "Fractions 1, 2, and 3"* are presented in papers by Stull and associates.[53-55] "Fractions 1P and 1S" represent preparations of "Fraction 1," reprecipitated a varying number of times.

The standardization of antigens on the basis of protein units of nitrogen (P.N.U.) was accomplished by Kjeldahl nitrogen determinations on the phosphotungstic acid precipitable substances.[56] One protein unit is equal to 0.00001 mg. or 0.01 microgram protein N. The dose of antigen was 0.1 ml. of any given concentration.

B. Storage of antigens: In early experiments, antigens were stored at refrigerator temperature 4 to 6° C., without further treatment, and aliquots were removed as required with sterile syringe and needle. Later work indicated the desirability of mixing antigen extracts with an equal volume of glycerin (Baker and Adamson), corroborating the findings of Hampton and associates[57] that glycerin aids in preventing the deterioration of the antigenic activity of ragweed extracts. Furthermore, when glycerin was added the preparation of antigen dilutions was facilitated since sterile precautions were no longer necessary in withdrawing antigen from storage bottles.

Immune Rabbit Serum.—

Alum-precipitated low-ragweed extract was prepared and used as follows: to 130.5 ml. of a sterile nonphenolized extract, 52.2 ml. of sterile alum solution (2.5 per cent $K_2SO_4.Al_2[SO_4]_3.24H_2O$) was added. Then 7.2 ml. of sterile 2 per cent NaOH was added slowly. The pH of an aliquot was 6.7 but since there was very little alum precipitate present, additional amounts of alum solution were added, 30 ml. in all, at which point the turbidity appeared adequate. The

*Donated by the Institute of Allergy, Roosevelt Hospital, New York, N. Y.

pH of the alum-precipitated ragweed extract was 4.5. An additional 7.0 ml. of 2 per cent NaOH produced a pH of 6.45. Finally 19.0 ml. of sterile physiologic saline was added. The original extract contained 920 micrograms protein N/ml.; the alum-precipitated ragweed extract contained approximately 500 micrograms protein N/ml. Rabbits were injected intramuscularly with approximately 10 mg. protein N. After 3 weeks, trial bleedings were made. When satisfactory evidence of antibody activity was obtained, the rabbits were exsanguinated by cardiac puncture. An additional dose of 1.25 mg. protein N. was given to those rabbits showing low antibody titers, and these animals were bled one week after injection. With this method all rabbits developed antibody, but only six out of eight gave usable titers. The serum was passed through Seitz filters previously washed with physiologic saline and was stored at –20° C. in aliquots suitable for testing. To minimize dehydration, test tubes were stoppered with paraffin-coated corks. Rabbit serum was heated at 56° C. for 30 minutes on the day of tests. Rabbit serum, stored as described, appeared to maintain antibody activity for over one year.

Hemolytic System.—

The hemolytic system employed was a modification of the method developed by the New York State Department of Health.[58, 59]

1. *Saline* (Veronal buffer):[60] Veronal buffer was substituted for ordinary saline. To prepare 4 liters of stock solution, 10.2 Gm. of barbituric acid, 2.0 Gm. of $MgCl_26H_2O$, and 0.4 Gm. of $CaCl_22H_2O$ were dissolved in about 1 liter of hot distilled water. In another flask, 167.6 Gm. of NaCl, 5.04 Gm. of $NaHCO_3$, and 5.0 Gm. of sodium barbiturate were dissolved in about 2.5 liters of distilled water. The two solutions were mixed and, when cool, diluted to 4 liters. For daily use, in the tests, one part of stock Veronal buffer was diluted with four parts of distilled water. The diluted buffer had a pH of 7.4.

2. *Sheep Cells:* Blood was collected from the external jugular into a preserving solution, containing sterile 3.8 per cent sodium citrate solution according to the method of Portnoy and associates.[61] Preserved cells were aged for at least 48 hours before being used. A 5 per cent suspension was prepared on the day of tests by washing preserved cells three times in 8-10 volumes of Veronal buffer. The cells were then transferred to a 15 ml. graduated centrifuge tube and centrifuged at 2,000 r.p.m. in a Type 1, SB International Centrifuge for 10 minutes. The cell volume was read, the supernatant removed, and a 5 per cent suspension prepared from the packed cells. 5.0 ml. of the 5 per cent suspension was placed in a 15 ml. graduated centrifuge tube and packed as before, in order to ascertain the accuracy of the suspension prepared and to provide packed cells for the preparation of the hemoglobin solution used in making the reading standards (Table I).

Reading of 70 per cent hemolysis or greater were made in suspension, whereas reactions giving less than 70 per cent hemolysis were centrifuged for several minutes, and the supernatants compared against a white background with centrifuged standards (from 0 to 65 per cent hemolysis).

3. *Hemolysin:*

a. *Preparation of stock 1-100 hemolysin:* Antisheep rabbit hemolysin preserved in an equal volume of glycerin was employed. A 1-100 stock dilution was prepared in the following manner:

1. 47.0 ml. of Veronal buffer.

2. 2.0 ml. of 5 per cent phenol in 0.85 per cent saline. (It was noted that a precipitate formed when 5 per cent phenol was made up in Veronal buffer.)

3. 1.0 ml. of hemolysin (50 per cent in glycerin).

Stock 1 to 100 solutions thus prepared were used over a period of one month.

TABLE I. PREPARATION OF READING STANDARDS

DEGREES OF HEMOLYSIS	2.5 PER CENT HEMOLYZED CELLS (ML.)*	2.5 PER CENT INTACT CELLS (ML.)†	VERONAL BUFFER
0	0	.2	.4
5	.01	.19	.4
10	.02	.18	.4
15	.03	.17	.4
20	.04	.16	.4
25	.05	.15	.4
30	.06	.14	.4
35	.07	.13	.4
40	.08	.12	.4
45	.09	.11	.4
50	.10	.10	.4
55	.11	.09	.4
60	.12	.08	.4
65	.13	.07	.4
70	.14	.06	.4
75	.15	.05	.4
80	.16	.04	.4
85	.17	.03	.4
90	.18	.02	.4
95	.19	.01	.4

*Prepared from cell pack recovered from 5.0 ml. of 5 per cent cells. After removing the supernatant, distilled water was added to the 9.0 ml. mark, the cells were laked by gentle inversion, and the resulting hemoglobin solution was made isotonic by the addition of 1.0 ml. of 8.5 per cent NaCl solution.

†Prepared by mixing an equal volume of 5 per cent cells with Veronal buffer.

b. *Determination of optimal hemolysin dilution:* Parallel twofold dilutions beginning with 1-250 and ending with 1-4,000 were prepared from the stock 1-100 hemolysin. To 0.1 ml. of each of these dilutions, 0.1 ml. of a dilution of guinea pig serum containing approximately one 50 per cent hemolytic unit, 0.2 ml. of Veronal buffer, and 0.1 ml. of 5 per cent sheep cells were added. After 15 minutes incubation at 37° C. water bath the degree of hemolysis was determined by comparison with the standards. That dilution of hemolysin, beyond which a further increase in concentration did not increase the degree of hemolysis, was selected as optimal. Likewise, 0.1 ml. of the hemolysin dilutions was tested for agglutinating activity by adding 0.1 ml. of 5 per cent cells and 0.4 ml. of saline; these test mixtures were incubated at 37° C. for 15 minutes, followed by refrigeration overnight. No agglutination was observed at the dilution found to be optimal for hemolytic activity and only a trace of agglutination was observed with four times the optimal concentration of hemolysin.

4. *Complement:* Both frozen and lyophilized guinea pig serum were used as complement. For frozen complement the sera of at least ten guinea pigs were pooled after exsanguination by cutting the neck vessels. Aliquots were stored at –20° C. and used over a period of 3 weeks. Lyophilized complement was obtained commercially from Carworth Farms, New City, N. Y., and Texas Biological Co., Fort Worth, Tex. Frozen and lyophilized complement proved equally satisfactory, although most of the work was done with the latter type.

Complement was titrated daily as follows: a 1-40 dilution was made in Veronal buffer employing 0.1 ml. of complement (pipetted with a 0.2 ml. pipette) and 3.9 ml. of saline. Varying amounts of this dilution were tested in duplicate according to the following scheme:

	TUBE NUMBER						
REAGENT ADDED	1	2	3	4	5	6	7
ml. 1-40 complement	.1	.07	.05	.04	.03	.02	0
ml. Veronal buffer	.3	.33	.35	.36	.37	.38	.4
ml. sensitized cells*	.2 to all tubes						

Incubation period, 15 minutes at 37° C., water bath.

*Sensitized cells were prepared by mixing 2.0 ml. of 5 per cent sheep-cell suspension and 2.0 ml. of optimal hemolysin dilution, pouring hemolysin onto cells, then back and forth a total of eight times. A minimum of ten minutes was allowed for sensitization at room temperature, prior to use. Sensitized cells were never used for longer than two hours after sensitization.

The degrees of hemolysis in the various tubes were determined by comparison with freshly prepared reading standards.

For the determination of the 50 per cent unit of complement the Van Krogh formula, $x = k\left(\frac{y}{1-y}\right)^{1/n}$ was employed in its logarithmic form, $\log x = \log k + 1/n \log \frac{y}{1-y}$, where x = the amount of complement for a given degree of hemolysis y, k is the amount of complement required for 50 per cent hemolysis, and 1/n is a parametric constant defining the slope of the line obtained by plotting log x versus $\log \frac{y}{1-y}$. Numerous titrations gave 1/n values of about 0.2. To facilitate the derivation of the 50 per cent unit, a chart (Table II) was prepared relating the values of y to $\frac{y}{1-y}$.

The values for x and $\frac{y}{1-y}$ were plotted directly on log paper and the 50 per cent unit was found by fitting a straight line through the experimental points and determining the amount of complement equivalent to the intercept of 1, i.e., where $y = 0.5$, $\frac{y}{1-y} = 1$. As required, dilutions containing one or more 50 per cent hemolytic units were prepared.

5. *Fixation time and temperature:* Unless otherwise noted the period of fixation of complement was 4 hours at a temperature of 3 to 6° C.

EXPERIMENTAL PROCEDURES AND RESULTS

Quantitative Complement Fixation Studies With Ragweed-Pollen Extracts and Homologous Rabbit Antisera.—

TABLE II. MATHEMATICAL RELATIONSHIP OF y TO $\frac{y}{1-y}$

VALUE OF y	VALUE OF $\frac{y}{1-y}$
0.05	0.05
0.10	0.11
0.15	0.18
0.20	0.25
0.25	0.33
0.30	0.43
0.35	0.54
0.40	0.67
0.45	0.82
0.50	1.00
0.55	1.22
0.60	1.50
0.65	1.86
0.70	2.33
0.75	3.00
0.80	4.00
0.85	5.67
0.90	9.00

1. *Titration of anticomplementary activity of antigens:* In Table III the effect of various types of antigen preparations on complement activity is presented.

TABLE III. THE ANTICOMPLEMENTARY EFFECT OF VARIOUS TYPES OF ANTIGEN PREPARATIONS

TYPE OF ANTIGEN PREPARATION*	MICROGRAMS OF ANTIGEN PROTEIN N ADDED									
	6.4	3.2	1.6	0.8	0.4	0.2	0.1	0.05	0.025	0
Whole extract				30	*40*	50	50	50	50	50
"Fraction 1"				10	30	*40*	40	50	50	
"Fraction 2"	0	20	25	*40*	40	45				
"Fraction 3"			0	0	*40*	45	50	50		
"Fraction 1P"†	0	10	30	*40*	55	50				
"Fraction 1S"†	0	15	30	*50*	40	45				

Results above expressed in degrees of hemolysis; 1 C′ unit added.
*All antigen preparations glycerinated.
†Serial twofold dilutions beginning with 5 micrograms of protein N.

The highest concentration of any given antigen preparation without significant anticomplementary effect has been underlined. No marked differences in this property were noted among the various antigens, although at least three different lots of each whole extract were examined. On the basis of these titrations, dilutions of antigens were selected which were not significantly anticomplementary in fixation reactions with rabbit immune serum.

2. *The fixation of complement by constant antibody in the presence of varying antigen concentrations:* Several pools were made of rabbit antisera to whole ragweed extract. By preliminary titration, the anticomplementary property of these pools was studied to select appropriate concentrations of immune serum for further testing with varying antigen concentrations.

In Table IV and Fig. 1 a typical protocol is presented. 0.1 ml. of antiserum, 0.1 ml. of whole extract antigen dilutions, 0.1 ml. of complement dilution, 0.1

TABLE IV. THE FIXATION OF COMPLEMENT (C′) BY CONSTANT ANTIBODY IN THE PRESENCE OF VARYING ANTIGEN CONCENTRATIONS

DILUTION OF IMMUNE SERUM	C′ UNITS ADDED	MICROGRAMS OF ANTIGEN PROTEIN N					
		0.8	0.4	0.2	0.1	0.05	0.025
1-30	3	*10	10	0	5	60	100
	6	30	0	0	25	100	100
	9	55	10	20	95	100	100
1-40	3	50	10	0	10	70	100
	6	85	20	15	55	100	100
	9	100	70	55	100	100	100
1-50	3	90	50	10	35	90	100
	6	100	95	50	90	100	100
	9	100	100	100	100	100	100
		Units of C′ required for 50 per cent hemolysis (based on maximal reactions).†					
1-30		8.8	11.5	10.6	7.2	2.7	
1-40		4.4	7.7	8.8	5.8	2.5	
1-50		1.9	3.0	6.0	4.0	1.9	

*Results expressed in degrees of hemolysis.
†For explanation of derivation of these values see Discussion.

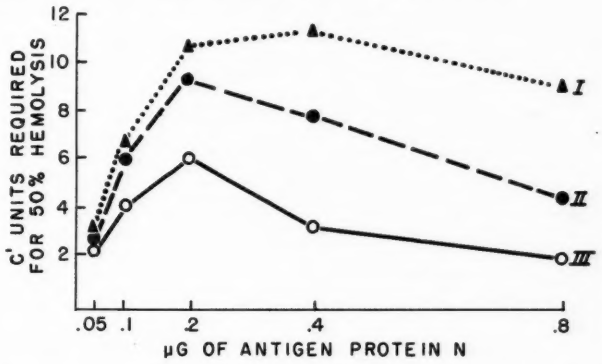

Fig. 1.—The fixation of complement by constant antibody in the presence of varying antigen concentrations:
Curve I. Rabbit immune serum, 1-30.
Curve II. Rabbit immune serum, 1-40.
Curve III. Rabbit immune serum, 1-50.

ml. saline were allowed to incubate 4 hours 3 to 6° C. Residual complement activity was then determined by adding 0.2 ml. of 5 per cent sensitized sheep red cells and incubating at 37° C. for 15 minutes.

From Fig. 1, it will be noted that with any given amount of antiserum, increasing fixation of complement occurs as the antigen concentration is increased to a maximum, and that beyond this maximum the amount of complement fixed decreases. It will also be noted from Table IV and Fig. 1, that with the highest concentration of antibody (1-30), the peak of fixation of complement occurred with 0.4 microgram of antigen protein N, whereas with smaller amounts of serum this peak occurred with 0.2 microgram of antigen protein N. Numerous experiments with whole extract antigens have shown that a concentration of 0.2 microgram of antigen protein N was optimal when 3 to 6 units of C′ were employed, whereas a concentration of 0.4 microgram of antigen protein N produced maximal fixation with 9 to 12 C′ units.

Limited studies of a similar nature with antigens "Fraction 1," "Fraction 1P," and "Fraction 1S" showed the same pattern of increasing fixation of C′ as the amount of antigen was increased to a maximum, then decreasing fixation as the antigen concentration was further increased.

3. *Comparative reactivity of immune rabbit sera and various pollen antigens:* Eleven individual rabbit sera from animals immunized to whole ragweed extract were tested in serial twofold dilutions, beginning with 1-5, against a single optimal dose of each of four antigens (whole extract antigen Lot "B," and "Fractions 1, 2, and 3") in the presence of six 50 per cent hemolytic units of complement. The fixation period was 18 hours at 6° C. The results obtained are presented in Table V. From the computed averages, the order of reactivity (with the most reactive first) of the different antigens was: whole extract, "Fraction 1," "Fraction 2," and "Fraction 3." With any single immune serum, the whole extract antigen was always more reactive than any of the antigen fractions. Of the fractions, "Fraction 1" was more reactive than "Fractions 2 and 3," except for antisera 188 and 190, where "Fraction 2" was slightly more reactive. In general, "Fraction 3" exhibited very slight reactivity.

In another experiment, a pool of immune rabbit sera, Lot 252, and five individual immune sera were tested against six different antigens. Three of these individual sera were prepared by immunizing against "Fraction 3," the others against the whole extract. The technique employed was similar to that described here, except that a four-hour incubation period, at 6° C. was used. The results obtained are given in Table VI and again illustrate the greater reactivity of whole ragweed extract in comparison to the various fractions. "Fractions 1P and 1S" were more reactive than "Fractions 1, 2, and 3." "Fraction 1P" was only slightly less reactive than the whole extract. Although "Fraction 3" showed little or no reactivity as a test antigen with all sera tested, immune sera 8, 9, and 11 prepared against "Fraction 3" were significantly reactive with whole extract, "Fractions 1, 1P, and 1S."

TABLE V. COMPARATIVE REACTIVITY OF ELEVEN IMMUNE RABBIT SERA AND VARIOUS POLLEN ANTIGENS (FIXATION PERIOD 18 HOURS, 6° C.)

	POLLEN ANTIGENS TESTED AT OPTIMAL DILUTIONS			
IMMUNE SERUM NO.	WHOLE EXTRACT	"FRACTION 1"	"FRACTION 2"	"FRACTION 3"
9	60	30	0	10
11	20	15	0	0
15	30	15	10	0
182	160	120	30	0
183	5	5	0	0
185	60	30	30	0
186	60	30	15	10
188	60	30	60	0
189	5	5	0	0
190	30	15	20	0
193	80	30	30	0
Averages	52	30	18	2

Results above expressed as the reciprocal of the highest serum dilution (interpolated) giving 50 per cent hemolysis with six units of complement.

TABLE VI. COMPARATIVE REACTIVITY OF IMMUNE RABBIT SERA OBTAINED BY INJECTIONS OF WHOLE EXTRACT OR "FRACTION 3" IN TESTS WITH WHOLE EXTRACT ANTIGENS AND SPECIAL "FRACTIONS"

IMMUNE SERUM	ANTIGEN USED IN IMMUNIZATION	ANTIGENS USED IN TESTS					
		1	2	3	4	5	6
Pool	Whole extract	40	30	5	0	30	30
2	Whole extract	60	40	5	0	30	40
4	Whole extract	120	40	5	0	120	50
8	"Fraction 3"	60	40	0	5	60	50
9	"Fraction 3"	80	60	0	0	80	60
11	"Fraction 3"	30	10	0	0	20	10
	Averages	65	37	2	1	57	40

Results above expressed as the reciprocal of the highest serum dilution (interpolated) giving 50 per cent hemolysis with six units of complement.

1.—Whole ragweed extract "Lot 2G"
2.—"Fraction 1"
3.—"Fraction 2"
4.—"Fraction 3"
5.—"Fraction 1P"
6.—"Fraction 1S"

DISCUSSION

Because of the difficulties experienced by previous investigators[62, 63] in using the complement fixation reaction to study the activity of pollen extracts presumably due to deterioration of antigen, over relatively short periods of time, it was apparent that some suitable method for preserving pollen extracts would be necessary. The demonstration of the effectiveness of glycerin in preserving antigenic activity of pollen extracts by Hampton and associates[57] prompted the use of this method for these studies. Experience with two lots of whole extract antigen, preserved in 50 per cent glycerin, has indicated that antigenic activity in complement fixation tests is maintained for at least one year.

It is evident from certain findings (Table III) that the ragweed antigens used in this study had marked anticomplementary properties which narrowed the range over which they could be employed.

In titrating varying antigen dilutions from time to time, variations of a minor sort were observed. The extent of these variations can be visualized from Table VII with reference to antigens "C" and "2G" which are whole extract antigens. Moreover, the similarity in fixation pattern of these lots of antigen can also be seen from Table VII. Based on the protein unit assay of three lots of whole extract antigen, antigen dilutions containing twenty protein units or 0.2 microgram protein N in the test dose were optimal with three to six complement units.

The values for the units of complement required for 50 per cent hemolysis (Table IV and Fig. 1) were derived from conversion factors according to the method used by the New York State Department of Health.[58] Titrations performed with the ragweed system indicated essential agreement with the factors obtained[58] in the system used for Wassermann antibody. For this reason it seemed appropriate to use the published[58] tables. The curve describing the fixation of complement by the pollen antigen-antibody system (Fig. 1) closely resembled the curve of precipitation described by Bukantz and

TABLE VII. REPRODUCIBILITY OF TEST RESULTS WITH TWO LOTS OF WHOLE EXTRACT ANTIGEN ON FOUR DIFFERENT TESTING DAYS

SERUM CONTROLS*			MICROGRAMS OF ANTIGEN PROTEIN N										TEST C′ UNITS ADDED
TESTING C DAY†	UNITS ADDED		ANTIGEN "LOT C"					ANTIGEN "LOT 2G"					
	1	2	0.8	0.4	0.2	0.1	0.05	0.8	0.4	0.2	0.1	0.05	
1	30	95	30	0	0	10	65	15	0	0	15	85	3
			80	5	20	90	100	60	0	20	95	100	6
			100	60	80	100	100	75	60	85	100	100	9
2	20	95	20	0	0	0	50	5	0	0	0	60	3
			90	10	10	55	100	80	10	10	85	100	6
			100	70	85	100	100	90	50	85	100	100	9
3	30	90						20	0	0	0	25	3
								60	15	5	50	100	6
								100	30	70	100	100	9
4	20	85	20	0	0	5	55						3
			40	10	15	70	100						6
			75	50	60	95	100						9

Results above expressed in degrees of hemolysis.
*Immune serum 1-30 dilution tested.
†Days not consecutive.

associates[25] for the same immune system, showing increased reactivity as the antigen concentration approached an optimum, then decreasing reactivity as the amount of antigen was increased beyond this optimal zone. Whereas Bukantz and associates[25] obtained maximal precipitation with 5,000 protein nitrogen units per milliliter, only 20 to 40 protein nitrogen units per 0.1 ml. were required for maximal fixation under the conditions of the studies herein described. Similar curves of reactivity for the fixation of complement were obtained by Osler and associates[64] using the pneumococcus Type III specific polysaccharide and homologous rabbit antibody, by Osler and Heidelberger[65] using chicken and duck egg albumin and rabbit antisera, and by Wallace and associates[66] using crystallized bovine serum albumin and homologous rabbit antibody. The comparison made with the latter papers[64-66] is not intended to imply that the ragweed antigens used were pure substances. Indeed, the data indicate that ragweed antigens were quite heterogeneous. However, the complement fixation reaction appears to be a sensitive tool for the detection of serologic activity of ragweed antigens since as little as 5 protein units or 0.05 microgram protein N was sufficient to produce a discernible fixation of complement (two to three 50 per cent units of C′). Osler and associates[64] noted that fixation of complement occurred with as little as 0.063 microgram of SIII and proposed that estimation of amounts of SIII below 1.0 microgram could be determined by use of a known amount of antibody previously calibrated. No studies were made along similar lines in this investigation because of the heterogeneity of the ragweed antigens and presumably of the corresponding antibodies. Conceivably, with improved methods for purifying antigens and for the preparation of suitable antisera, the complement fixation reaction, in view of its sensitivity, may prove to be a useful tool in assaying antigens for their various components.

Studies of the comparative reactivity of rabbit antisera and several types of pollen antigens showed that whole extract antigen consistently gave greater

fixation of complement than "Fractions 1, 2, and 3" (Table V). This might be merely a summation effect, resulting from a greater multiplicity of antigenic components and their corresponding antibodies. Of the three fractions, "Fraction 1" appeared to be most reactive. This would indicate either that this fraction was a more effective immunizing agent or that it had a greater capacity to fix complement in reacting with antibody. Similar reactive capacities of these different antigen preparations were noted in another experiment (Table VI).

In spite of the fact that "Fraction 3" was relatively weak as a test antigen, significant reactions were obtained with antisera prepared against this fraction in tests with whole extract antigen and "Fraction 1" antigen (Table VI). This would suggest the presence of antigenic impurities in "Fraction 3" which were capable of stimulating the production of antibodies, even though "Fraction 3" appears to be a poor test antigen. Furthermore, it can be seen from Table VI that "Fractions 1P and 1S" were also effective test antigens, while "Fraction 1P" appeared to be slightly more reactive than "Fraction 1S."

With regard to the antigenicity of these fractions, it is of interest that Sherman and Stull and associates[53-55] had noted "Fraction 1" to be allergenically more reactive than the other fractions in skin tests performed by the passive transfer method with serum from human cases sensitive to ragweed. Thus, of 200 ragweed-sensitive cases, using "Fraction 1" antigen, 188 were positive, 9 were doubtful, and 3 negative. With "Fraction 2," 82 were positive, 23 were doubtful, and 95 negative. Of the serums 155 gave larger reactions to "Fraction 1" than to "Fraction 2," 8 gave larger reactions to "Fraction 2," and 37 gave essentially similar reactions. These results were interpreted as evidence for the independent specificity of these two fractions.[55] With "Fraction 3," tests on 144 sera were made. Of these, 18 were positive, 6 doubtful, and 120 negative. The authors[55] concluded from these findings that "Fraction 3" was relatively unimportant in ragweed allergy. Further proof of the greater antigenicity of "Fraction 1" was the observation[55] that ragweed-sensitive cases treated with "Fraction 1" developed blocking antibodies to this fraction in most instances. Patients injected with "Fraction 2" developed antibodies to "Fraction 2," but the percentile response was not as great as was the antibody response to "Fraction 1." Patients injected with whole ragweed extract developed antibodies more readily to "Fraction 1" than to "Fraction 2."

SUMMARY AND CONCLUSIONS

1. Ragweed antigens preserved in an equal volume of glycerin and stored at refrigerator temperature were stable for at least one year when employed in complement fixation tests. Rabbit immune sera stored at –20° C. retained their potency for more than one year.

2. Ragweed antigens showed marked anticomplementary properties which restricted their use to a narrow range of concentration.

3. Within the range of antigen concentration studied, a curve of reaction was obtained which showed that antibody fixed increasing amounts of com-

plement as the concentration of antigen was increased to a maximum, beyond which inhibition of fixation occurred.

4. With three lots of whole extract antigen, a dose equivalent of 0.2 microgram protein N was optimal when three to six C′ units were employed.

5. Whole extract antigen gave greater fixation of complement than the special fractions studied. Of the fractions, "Fraction 1" was most reactive. Although "Fraction 3" was a poor test antigen, rabbits immunized with this "Fraction" showed considerable fixation of complement in the presence of whole extract antigen and "Fraction 1."

6. Because of the sensitivity of the complement fixation reaction (as little as 0.05 microgram protein N produced a discernible fixation of C′), and the stability of glycerin-preserved antigens, the complement fixation test may prove to be of value in the purification and standardization of ragweed extracts.

References given at the end of the following paper.

COMPLEMENT FIXATION STUDIES IN RAGWEED ALLERGY

II. Determination of Antibody in Human Sera to Ragweed Antigen by Means of a Complement Fixation Inhibition Test; the Relationship of Antibody So Determined to the Passive Transfer for Blocking Antibody

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INTRODUCTION

IN the preceding paper a study of the complement fixation reaction of ragweed antigen and rabbit immune serum was reported, which served as a background for experiments undertaken to develop a test for antibodies occurring in human ragweed allergy.

Preliminary observations by the authors indicated that inhibition of the fixation of complement (C′) by a standardized ragweed antigen-rabbit immune serum occurred with sera known to contain blocking antibody by passive transfer. The present report is concerned with a study of the factors influencing the detection of such inhibiting antibodies, culminating in the development of a test method for these antibodies. This test method was then applied to a study of allergic and nonallergic sera and comparisons were made with the passive transfer technique.

MATERIALS AND METHODS

1. *Human Sera.*—Unless otherwise noted, whenever blood or serum samples were received the serum obtained was passed through Seitz filters, 30 ml. capacity and then stored at -20° C. in aliquots suitable for testing. Paraffined corks were employed to minimize dehydration during storage. On the day of examination, sera were heated at temperatures and for periods of time as described under the particular protocol.

2. *Hemolytic System.*—These are described in Part I of these studies.

3. *Antigens, Rabbit Immune Serum.*—These are described in Part I of these studies.

4. *Complement Fixation Inhibition Test for Antibodies Against Ragweed Antigens.*—See description under experimental results and procedures.

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EXPERIMENTAL PROCEDURES AND RESULTS

A. *The Effect of Various Factors on the Detection of Inhibiting Antibody.*—

1. *The possible role of thermostable complement components:* By employing amounts of complement designed to produce partial hemolysis, it was shown that all the human sera tested, whether they possessed or were lacking in inhibiting antibody, did not enhance the hemolytic activity of added guinea pig complement. Thus, in one experiment when 16 fresh sera were tested, after heating at 56° C. for 30 minutes, with one 50 per cent hemolytic unit of C′, 15 to 40 per cent hemolysis occurred with an average of 27 per cent. These same sera heated at 56° C. for 4 hours produced 5 to 30 per cent hemolysis in the presence of one unit of complement with an average of 13 per cent hemolysis. When stored sera were examined for this property they showed in general less hemolysis than the fresh sera. As further experiments were performed and as a testing method was evolved, there was never any indication of a thermostable complement effect.

2. *The effect of preliminary incubation of serum and antigen at various temperatures for varying periods of time in the presence and absence of comple-*

TABLE I. THE EFFECT OF TIME, TEMPERATURE, AND THE PRESENCE OR ABSENCE OF COMPLEMENT DURING THE PRELIMINARY INCUBATION OF ANTIGEN AND HUMAN SERUM ON THE DETECTION OF INHIBITING ANTIBODY

			INHIBITION TEST‡ SERUM DILUTIONS†						ANTICOMPLEMENTARY TEST§ SERUM DILUTIONS†					
SYSTEM	SERUM	ANTIG.*	2	4	8	16	32	64	2	4	8	16	32	64
1	Neg.	.4	15	15	15	15	15	10						
	Pool	.2	15	15	15	15	15	15	5	15	30	55	70	75
	Block.	.4	50	60	50	20	10	5						
	Pool	.2	50	70	75	60	30	10	20	35	50	70	75	75
2	Neg.	.4	10	10	15	15	10	10						
	Pool	.2	5	10	20	20	15	10	5	15	30	50	70	75
	Block.	.4	55	70	65	40	25	15						
	Pool	.2	60	75	80	65	50	20	20	30	60	60	70	75
3	Neg.	.4	10	10	10	10	5	5						
	Pool	.2	10	10	10	10	5	5	10	15	30	45	60	65
	Block.	.4	40	60	35	15	5	5						
	Pool	.2	55	65	65	40	10	5	10	30	50	55	60	65
4	Neg.	.4	10	10	5	0	0	0						
	Pool	.2	5	10	10	0	0	0	10	10	20	30	50	55
	Block.	.4	40	55	40	10	0	0						
	Pool	.2	40	60	55	20	5	0	10	25	35	40	40	40

Results above expressed in degrees of hemolysis.

*Amounts given in micrograms of protein N.

†Reciprocal of serum dilutions.

‡Inhibition test with three units of C′.

§Anticomplementary test with two units of C′ (rabbit immune serum added).

Immune rabbit serum dilution used required approximately six units of C′ for 50 per cent hemolysis.

System 1.—Human serum plus antigen incubated 1 hour 37° C., then immune serum and C′ added.

System 2.—Human serum plus antigen plus C′ incubated 1 hour 37° C., then immune rabbit serum added. C′ dose based on titration using a preliminary incubation of 1 hour at 37° C. before adding sensitized cells.

System 3.—Human serum plus antigen incubated 18 hours 5° C., then immune serum and C′ added.

System 4.—Human serum plus antigen plus C′ incubated 18 hours 5° C., then immune rabbit serum added.

ment: Table I presents a representative protocol showing how time, temperature, and the presence of complement during the preliminary incubation of inhibiting antibody and antigen influenced the detectability of this antibody. To perform this experiment, dilutions of antigen "C" (whole extract antigen) were prepared to contain 0.4 and 0.2 microgram of antigen protein N. in a test dose of 0.1 ml. As a source of inhibiting antibody, a pool of three human sera was employed. The donors of these sera were individuals who were sensitive to ragweed and who had been treated with ragweed extract. Tests on the sera individually indicated the presence of inhibiting substance. For use in this and many other experiments, the three sera were Seitz-filtered, pooled, and stored at –20° C. until used in tests. Before testing, this pooled serum was heated at 56° C. for four hours. The dose of serum was 0.1 ml. of the dilutions indicated in Table I. Parallel tests were made on a pool of five individual sera selected at random from specimens submitted for serologic tests for syphilis, all of which had given negative results in several types of serologic tests. This negative serum pool served as an indicator for the specificity of the test for inhibiting antibody under the various conditions described. Test dosages of serum and antigen were pipetted and the mixtures incubated in the presence or absence of complement, as noted in Table I. The complement dose was three 50 per cent hemolytic units. Following this preliminary incubation, a pool of rabbit antiragweed immune serum was added in a test dose of 0.1 ml. of a 1-20 dilution. After 4 hours at 3 to 6° C., 0.2 ml. of sensitized sheep red blood cells was added and the degree of hemolysis recorded following 15 minutes' incubation at 37° C. Antigen, complement, and cell controls were included.

Following the recommendation of Rice,[43] 50 per cent or greater hemolysis with a test mixture was considered as evidence of inhibiting antibody action. Accordingly, no inhibiting activity was noted with the negative pool, whereas evidence of inhibition was obtained under the varying conditions of this experiment with the inhibiting antibody pool. The end point of reactivity of the inhibiting serum in any given system was always greater with the lower concentration of antigen. Using a 1-hour preliminary incubation at 37° C., there was a slight increase in reactivity endpoint when complement was present in the mixture as compared to the system lacking in complement. Prolonged incubation at 5° C. with and without complement produced lower end points than the shorter incubation at 37° C. The results given under serum controls were typical. The anticomplementary property of the serums tested can be noted. There was no significant difference in this property of the two serum pools studied.

It is apparent from this and similar experiments that preliminary incubation at 37° C. gave more sensitive findings than incubation at 5° C., and that the effect of complement during this preliminary incubation was negligible.

As extension of these time studies, in several experiments, assay of inhibiting antibody was made using 1 and 2 hours of preliminary incubation at 37° C. and 5° C. at the same time tests were made without preliminary incubation. The results obtained are given in Table II. It can be seen that evidence of inhibiting activity occurred even when immune rabbit serum was added

without preliminary incubation of antigen and inhibiting serum. A fourfold increase in titer of inhibiting serum occurred after 1 hour at 37° C. Further incubation at 37° C., i.e., 2 hours' total incubation, did not produce any more reactivity than 1 hour at 37° C. Results obtained at 5° C. were of lower magnitude. The individual serum No. 3, selected at random from sera-giving negative serologic test for syphilis, gave no evidence of inhibiting antibody, as defined, under all conditions of this experiment.

TABLE II. THE EFFECT OF 0, 1, AND 2 HOURS OF PRELIMINARY INCUBATION OF HUMAN SERUM AND ANTIGEN AT 37° C. AND 5° C. ON THE REACTIVITY OF INHIBITING ANTIBODY

			INHIBITION TEST* SERUM DILUTIONS‡					ANTICOMPLEMENTARY TEST† SERUM DILUTIONS‡				
SERUM	TIME	TEMP.	4	8	16	32	64	4	8	16	32	64
B.A.	0	Room	85	45	10	0	0	60	80	85	90	90
3	0	Room	15	5	0	0	0	85	85	90	90	95
B.A.	1	37° C.	95	90	60	10	0	65	80	85	90	90
3	1	37° C.	45	10	0	0	0	80	85	90	90	90
B.A.	1	5° C.	95	90	40	5	0	65	80	80	90	90
3	1	5° C.	25	5	0	0	0	80	90	90	90	90
B.A.	2	37° C.	95	90	70	20	0	65	80	80	90	90
3	2	37° C.	40	10	0	0	0	80	90	90	90	90
B.A.	2	5° C.	95	90	40	10	0	70	80	90	90	90
3	2	5° C.	40	10	0	0	0	85	90	90	90	90

Results above expressed in degrees of hemolysis.
Time given in hours.
B.A. represents an inhibiting antibody pool.
3 represents a serum, negative in serologic tests for syphilis.
*Inhibition test performed with 0.2 microgram of antigen protein N., three units of C′, and a dilution of immune rabbit serum which required six units of C′ for 50 per cent hemolysis.
†Anticomplementary test performed with two units of C′ (rabbit immune serum added).
‡Reciprocal of serum dilutions.

In other experiments it was shown that shaking up to 60 minutes at room temperature in a mechanical shaker did not augment the reactivity of inhibiting antibody. Furthermore, incubation at room temperature without shaking did not produce greater reactivity than at 37° C.

3. *The effect of antigen and immune serum concentration:* To determine the effect of antigen and immune serum concentration on the detection of inhibiting antibody, a preliminary exposure of 1 hour at 37° C. was used, since this time and temperature appeared optimal from previous experiments. A blocking antibody pool and a serum selected at random from sera negative serologic tests for syphilis were employed as test sera. Sera were heated at 56° C. for 4 hours. Serial twofold dilutions of the sera, beginning with 1-4, were tested against several dilutions of ragweed-whole-extract antigen. After preliminary exposure of antigen and serum, immune rabbit serum was added in two dilutions, and complement in four dilutions containing three, four, six, and eight units of C′. Test mixtures together with suitable controls were incubated at 5° C. for 4 hours. Sensitized cells were added and the degree of hemolysis was determined after 15 minutes' incubation at 37° C. water bath. Table III depicts the complement fixation pattern of the rabbit immune serum.

Table IV gives the results obtained in tests for inhibition. With the smallest amount of rabbit serum, i.e., 1-40, inhibitory action was observed with both a blocking pool and the normal serum used. With the 1-30 dilution of rabbit immune serum, specific results were obtained with three units of C′. Larger amounts of C′ gave nonspecific findings with the normal serum. Using the 1-20 dilution of rabbit immune serum, specific results were obtained with three and four units C′, whereas larger amounts of C′ produced nonspecific findings. With respect to antigen concentration it was noted that, under the conditions where specificity was maintained, more sensitive findings were obtained with the smallest amount of antigen, i.e., 0.2 microgram of protein N.

TABLE III. THE TITRATION OF RABBIT IMMUNE SERUM POOL (IS)

IMMUNE SERUM DILUTION	SERUM CONTROLS C′ UNITS 1	2	COMPLEMENT FIXATION RESULTS WITH MICROGRAMS OF ANTIGEN PROTEIN N. C′ UNITS	0.8	0.4	0.2	0.1	0.05	0.025
1-20	15	85	3	0	0	0	0	30	90
			4	0	0	0	5	80	100
			6	0	0	0	40	100	100
			8	0	0	0	60	100	100
1-30	25	90	3	20	0	0	0	60	95
			4	20	0	0	10	90	100
			6	40	0	0	50	100	100
			8	50	5	10	90	100	100
1-40	35	95	3	70	10	0	10	75	100
			4	75	20	10	20	100	100
			6	95	55	20	70	100	100
			8	95	85	65	95	100	100

Results above expressed in degrees of hemolysis.

TABLE IV. THE EFFECT OF ANTIGEN, RABBIT IMMUNE SERUM, AND COMPLEMENT CONCENTRATION ON THE DETECTION OF INHIBITING ANTIBODY

SERUM	IS DILUTION	INHIBITING ANTIBODY TITERS OBTAINED WITH 3 UNITS C′ 0.2	0.4	4 UNITS C′ 0.2	0.4	6 UNITS C′ 0.2	0.4	8 UNITS C′ 0.2	0.4
B.A.	1-20	8	4	8	8	8	4	16	8
Normal		0	0	0	0	4	0	8	4
B.A.	1-30	16	16	16	8	32	8	32	16
Normal		0	0	4	4	16	8	16	8
B.A.	1-40	32	32	32	>	>	>	>	>
Normal		8	16	16	>	32	>	>	>

Results above expressed in terms of the reciprocal of the highest serum dilution showing evidence of inhibition; 0 indicates no inhibition.

0.2 and 0.4 refer to the microgram of antigen protein N. used.

> represents reactions greater than 128.

4. *The effect of time and temperature of inactivation:* Sera were exposed to various temperatures for varying times in a water bath. Following this, tests for inhibiting antibody were made using a preliminary exposure of 1 hour at 37° C. for mixtures of test sera in the presence of 0.2 microgram of antigen protein N. Rabbit immune serum was added in a dilution of 1-30, plus three units of C′, and the test was completed as previously described. The results are given in Table V. No tests were made in those instances where a serum aliquot showed coagulation at a particular exposure. For this reason

TABLE V. THE EFFECT OF TIME AND TEMPERATURE OF HEATING SERA ON THE DETECTION OF INHIBITING ANTIBODY

SERA	HEATING TIME	HEATING TEMP.	RESULTS OF INHIBITION TEST	CLINICAL STATUS
CF	0.5	56	Pos. 1-2	Normal, nonsensitive
	1	56	Neg.	
	2	56	Neg.	
	4	56	Neg.	
	0.5	60	Neg.	
5023	0.5	56	Neg.	Ragweed-sensitive, untreated
	1	56	Neg.	
	2	56	Neg.	
	4	56	Neg.	
	0.5	60	Neg.	
	0.5	64	Neg.	
5026	0.5	56	Pos. 1-8	Ragweed-sensitive, ragweed-treated
	1	56	Pos. 1-16	
	2	56	Pos. 1-16	
	4	56	Pos. 1-16	
	0.5	60	Pos. 1-16	
	0.5	64	Pos. 1-8	

Inhibition test with 0.2 microgram of antigen protein N.; three units of C'.
Results expressed as highest serum dilution showing evidence of inhibition.
Time in hours; temperature in centigrade.

no tests were made on serum CF exposed to temperatures of 64°, 68°, and 72° C.; on sera 5,023 and 5,026 exposed to temperatures of 68° and 72° C.

It can be seen from Table V that serum CF, from a normal nonsensitive donor, gave only a minimum reaction, i.e., 1-2, with an aliquot heated at 56° C. for 30 minutes. Negative reactions were obtained at other times and temperatures indicated. Serum from a ragweed-sensitive donor (5,023) gave negative reactions throughout. Evidence of inhibiting antibody was obtained with serum 5,026, donated by a ragweed-sensitive, ragweed-treated individual, with all aliquots tested. Thus, for example, an aliquot heated at 56° C. for 30 minutes produced an endpoint of 1-8 serum dilution. This serum heated for 1, 2, and 4 hours at 56° C. gave identical endpoints of 1-16. An aliquot heated at 64° C. gave an endpoint of 1-8.

5. *The effect of inhibiting antibody and sensitizing antibody on the fixation of C' by rabbit antibody over a range of antigen concentration:* Experiments to show the effect of antigen and immune-serum concentration on the detection of inhibiting antibody have been previously described under item 3 of this series. Since a curve of reactivity for rabbit antibody in the presence of ragweed extracts had been established (see Fig. 1 of Part I) experiments were designed to show what effect human sera containing inhibiting antibody and sensitizing antibody (by passive transfer) might have on this relationship. Two lots of whole-extract antigen were studied. Antigen dilutions were prepared to contain 0.8, 0.4, 0.2, 0.1, 0.05, and 0.025 microgram of antigen protein N. in the test dose of 0.1 ml. The following human sera were studied: B.A., representing a pool of three donors, ragweed sensitive and ragweed treated; 5,034 representing a ragweed-sensitive, untreated patient, containing skin-sensitizing antibody by passive transfer. B.A. serum was heated at 56° C. for 4 hours, whereas aliquots of 5,034 were heated at 56° C. for ½ and 4 hours.

Tests were made using a preliminary exposure of 1 hour at 37° C. of the human sera and antigen dilutions. Then immune rabbit serum and three units of complement were added. Tests were incubated at 5° C. for 4 hours. Determinations of residual complement activity were made as previously described. The amounts of complement required for 50 per cent hemolysis were determined by reference tables provided in *Standard Methods.*[58] These values were plotted against the amounts of antigen added (Figs. 1 and 2). Since the results obtained with both lots of antigen were essentially the same, the findings with only one lot of antigen are given. With the serum (B.A.) containing blocking antibody by passive transfer, evidence of inhibition was obtained

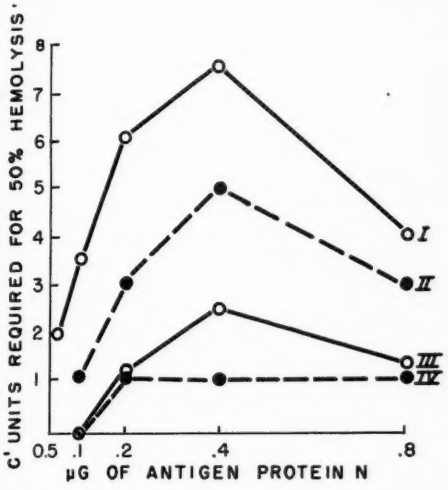

Fig. 1.

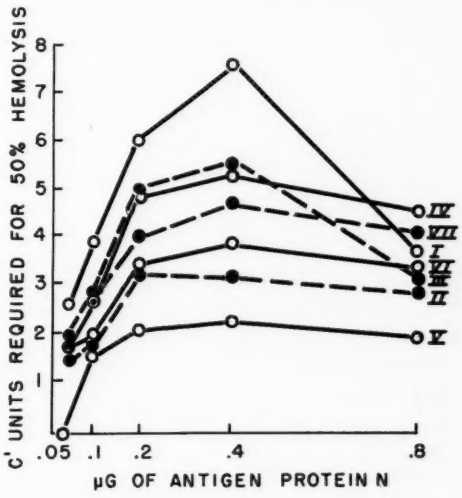

Fig. 2.

Fig. 1.—The effect of human serum containing blocking antibody by passive transfer on the course of the reaction of whole pollen extract and rabbit-immune serum. Curve I. Rabbit-immune serum, 1-30. Curve II. Blocking antibody serum heated 4 hours 56° C. (1-64 dilution). Curve III. Blocking antibody serum heated 4 hours 56° C. (1-16 dilution). Curve IV. Blocking antibody serum heated 4 hours 56° C. (1-4 dilution).

Fig. 2.—The effect of human serum containing sensitivity antibody by passive transfer on the course of the reaction of whole pollen extract and rabbit-immune serum. Curve I. Rabbit-immune serum 1-30. Curve II. Serum 5,034 heated ½ hour 56° C. (1-4 dilution). Curve III. Serum 5,034 heated ½ hour 56° C. (1-16 dilution). Curve IV. Serum 5,034 heated ½ hour 56° C. (1-64 dilution). Curve V. Serum 5,034 heated 4 hours 56° C. (1-4 dilution). Curve VI. Serum 5,034 heated 4 hours 56° C. (1-16 dilution). Curve VII. Serum 5,034 heated 4 hours 56° C. (1-64 dilution).

with the 1-4 and 1-16 dilutions according to the criterion of 50 per cent or greater hemolysis. With serum 5,034 containing sensitizing antibodies by passive transfer, the aliquot heated at 56° C. for ½ hour showed no inhibition according to the established criterion. However, a depressant action (though much weaker than that obtained for B.A. serum) on the fixation curve of the rabbit immune serum can be seen. The aliquot of 5,034 heated for 4 hours at 56° C. showed more of an inhibitory effect than the aliquot heated for only 30 minutes. By using the criterion for evidence of inhibiting antibody as previously described, inhibtory action was noted only with the 1-4 dilution of the aliquot of 5,034 heated for 4 hours.

6. *Comparative reactivity of inhibiting human serum against whole-ragweed-extract antigen and several special fractions of ragweed antigen:* During

the course of these investigations it was deemed of interest to study the behavior of inhibiting substances found in some ragweed-treated patients against whole-extract antigen in the presence of other ragweed fractions. Because of limited supplies of appropriate sera and special antigens, only one experiment was made. The conditions of the experiment were similar to those described under the preceding section 5. Since only Fractions "1," "1P," and "1S" gave satisfactory titers with the immune-rabbit-serum pool, studies were made with these only. All antigens were tested in a single predetermined optimal dose. Four individual sera were tested after heating at 60° C. for ½ hour. CF represents a normal donor; 5,023, a ragweed-sensitive case; and 5,026, a ragweed-sensitive treated patient. The fourth serum studied was a normal rabbit pool. The results obtained are presented in Table VI. If the criterion of 50 per cent or greater hemolysis as evidence of inhibition is used, it can be seen that only serum 5,026 showed inhibitory action. The inhibition was observed with all four antigens. The serum titer with antigen "Lot 2G" was 1-16, with antigen "FR. 1" it was 1-32, with antigen "FR. 1P" it was 1-16, and with antigen "FR. 1S" it was 1-32.

7. *The effect of varying the incubation period following the addition of rabbit-immune serum on the detection of inhibiting antibody:* In early experiments it seemed desirable to use an overnight incubation period rather than the 4-hour period to facilitate the analyses for inhibiting antibody. Upon performing assays for inhibiting antibody with the longer period of incubation, it was found that inhibiting action could not be detected. A possible explanation for this failure in detecting antibody seemed to be a reversal phenomenon, whereby

TABLE VI. THE COMPARATIVE REACTIVITY OF INHIBITING HUMAN SERUM AGAINST WHOLE-RAGWEED-EXTRACT ANTIGEN AND SPECIAL FRACTIONS "1, 1P, AND 1S"

SERUM	ANTIGEN	TEST WITH THREE UNITS C′ SERUM DILUTIONS* 2	4	8	16	32	64	INHIBITION TITER
CF	Whole Extr.	0	0	0	0	0	0	Neg.
	"Fract. 1"	15	5	0	0	0	0	Neg.
	"Fract. 1P"	0	0	0	0	0	0	Neg.
	"Fract. 1S"	5	0	0	0	0	0	Neg.
5023	Whole Extr.	20	20	10	0	0	0	Neg.
	"Fract. 1"	45	40	20	10	0	0	Neg.
	"Fract. 1P"	10	25	0	0	0	0	Neg.
	"Fract. 1S"	25	15	0	0	0	0	Neg.
5026	Whole Extr.	40	75	80	70	20	5	Pos. 1-16
	"Fract. 1"	35	70	80	80	60	20	Pos. 1-32
	"Fract. 1P"	40	70	75	70	30	0	Pos. 1-16
	"Fract. 1S"	30	60	75	70	55	5	Pos. 1-32
NRS	Whole Extr.	5	15	0	0	0	0	Neg.
	"Fract. 1"	35	15	10	0	0	0	Neg.
	"Fract. 1P"	5	5	0	0	0	0	Neg.
	"Fract. 1S"	15	5	0	0	0	0	Neg.

Results under test expressed in degrees of hemolysis.
Whole extract—dose used 0.2 microgram protein N.; immune serum dilution 1-40.
"Fraction 1"—dose used 0.2 microgram protein N.: immune serum dilution 1-20.
"Fraction 1P"—dose used 1.25 microgram protein N.; immune serum dilution 1-30.
"Fraction 1S"—dose used 1.25 microgram protein N.; immune serum dilution 1-30.
Inhibition titer expressed as highest serum dilution showing inhibition.
*Reciprocal of serum dilutions.

bound antigen was released from inhibiting antibody and then recombined with rabbit-immune serum. An experiment was designed to test this hypothesis, employing a serum containing inhibiting antibody by passive transfer, 5,026, and a normal serum, CF. After heating the sera at 60° C. for ½ hour, 1-8 dilutions of each were prepared. Aliquots of 0.1 ml. were incubated at 37° C. for 1 hour with 0.2 microgram protein N. of whole-extract antigen "2G." Following this preliminary exposure, five different dilutions of immune rabbit serum, and three 50 per cent C′ units were added. Tests of the immune rabbit serum, antigen, and complement were likewise made in addition to the other customary controls. Determinations of the amount of complement fixed were made at 1, 2, 2¾, 3½, 6, and 20 hours. For each time interval, the dilution of rabbit antiserum requiring 6 C′ units for 50 per cent hemolysis was estimated on the basis of the test findings with the dilutions actually tested. By using the criterion of 50 per cent or greater hemolysis as evidence of inhibitory activity, results were expressed, for the two human sera tested, as + indicating inhibition, or 0 indicating no inhibition. From Table VII it can be seen that the serum containing blocking antibody by passive transfer displayed inhibiting activity at every time interval when related to the titer to the rabbit-immune serum. As the incubation period was prolonged, i.e., at 6 and 20 hours, and the rabbit-immune serum fixed more complement, the blocking serum showed inhibitory activity in the presence of smaller amounts of rabbit-immune serum, but not with larger amounts of this serum. With the normal serum, at any given time interval, there was no evidence of inhibition when the results obtained were related to the immune-rabbit-serum reactivity. For example, after 1-hour incubation, the immune-rabbit-serum dilution requiring 6 C′ units for 50 per cent hemolysis was 1-5. The human serum tested in the presence of 1-10 rabbit serum was not inhibitory. After 3½ hours, the immune-rabbit-serum dilutions requiring 6 C′ units for 50 per cent hemolysis was 1-25. The normal human serum showed no inhibitory activity in the presence of 1-40 immune rabbit serum.

TABLE VII. THE EFFECT OF VARYING THE INCUBATION PERIOD FOLLOWING THE ADDITION OF RABBIT-IMMUNE SERUM ON THE DETECTION OF INHIBITING ANTIBODY

FIXATION PERIOD IN HOURS	HUMAN SERUM 5026 IMMUNE SERUM DILUTIONS*					HUMAN SERUM CF IMMUNE SERUM DILUTIONS*					IMMUNE SERUM TITER
	10	20	40	80	160	10	20	40	80	160	
1	+	+	+	+	+	0	+	+	+	+	5
2	+	+	+	+	+	0	0	+	+	+	10
2¾	+	+	+	+	+	0	0	0	+	+	15
3½	+	+	+	+	+	0	0	0	+	+	25
6	0	+	+	+	+	0	0	0	+	+	40
20	0	0	0	+	+	0	0	0	0	+	80

*Reciprocal of serum dilutions.
+Evidence of inhibition.
0 No inhibition.
Immune serum titer, reciprocal of interpolated dilution requiring six C′ units for 50 per cent hemolysis.

8. *The selection of a test method for the detection of inhibiting antibody:* Preliminary studies with a few selected cases known to contain blocking anti-

body by passive transfer indicated that evidence for inhibiting activity, presumably due to the presence of antibodies, could be detected in some instances by a complement fixation inhibition test. Moreover, noninhibitory activity with normal sera or ragweed-sensitive sera not containing blocking antibody by passive transfer suggested that the reaction might be specific under certain conditions. Attempts were made to increase the sensitivity of the test, in order to obtain a better correlation with the passive transfer results on these few selected cases. As the sensitivity was raised it appeared that the specificity decreased. To illustrate this effect a composite of several experiments is presented in Table VIII. The data indicated the marked improvement in specificity obtained by using an immune-rabbit-serum dilution of 1-20, which required approximately 9 units of C′ for 50 per cent hemolysis. Smaller amounts of rabbit antiserum gave less specific findings.

TABLE VIII. THE EFFECT OF IMMUNE RABBIT SERUM CONCENTRATION ON THE SENSITIVITY AND SPECIFICITY OF THE COMPLEMENT FIXATION INHIBITION TEST

SERUM NO.	IN VIVO TEST	INHIBITION TEST USING IMMUNE SERUM DILUTIONS		
		1-40	1-30	1-20
5039	0	8	8	0
5040	+	32	16	0
5043	+	16	16	8
5052	+	32	32	8
5059	+	16	8	0
5076	+	32	32	8
5035	0	16	8	0
5070	0	4	4	0

Inhibition test results expressed as the reciprocal of the highest serum dilution showing inhibition; 0 indicates no inhibition.

In vivo test, passive transfer test for blocking antibody; + indicates a positive reaction, 0 indicates a negative reaction.

Another consideration was the anticomplementary property of the sera submitted for assay. Unsuccessful attempts were made to abolish this property by preliminary treatment of the sera with active complement, according to the method of Taran.[67] As a working method, the test dose of complement was increased from three to four complement units.

The method ultimately selected for use in the assay of sera for inhibiting antibody is now described.

1. Saline-Stock Veronal Buffer, diluted on the same day the tests were made.

2. Sheep Red Cells, 5 per cent suspension, prepared daily. The test dose was 0.1 ml.

3. Complement-hemolytic activity was determined as previously described. Two dilutions were then prepared containing one and four 50 per cent hemolytic units in a test in dose of 0.1 ml.

4. Hemolysin-optimal dilution was determined as previously described. A stock 1-100 dilution was prepared as previously noted and was used for as long as 1 month. The test dose was 0.1 ml.

5. Sensitized sheep red blood cells, equal volumes of 5 per cent cells and optimal hemolysin dilution were mixed by pouring hemolysin on cells, then

back and forth for a total of eight excursions. This mixture was not used before 10 minutes, nor longer than 2 hours after preparation.

6. Reading Standards, these were prepared daily as previously described. For simplification, 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 per cent standards were used. In making readings of hemolysis it was advisable to chill the tubes by placing the racks in an ice water bath, since hemolysis will continue at a slow rate at room temperature after removal from the 37° C. water bath.

7. Immune Rabbit Serum, a pool of serum prepared from rabbits immunized against whole-extract-pollen antigen was employed. Preliminary assay to determine suitability of each individual serum was made. Suitable aliquots of immune serum were stored at –20° C. in Pyrex test tubes stoppered with paraffin-coated corks. For use in tests, aliquots were thawed, thoroughly mixed, and heated at 56° C. for 30 minutes. The dilution of immune rabbit serum for use in inhibition test contained an amount of antibody which required eight to ten units of C′ to produce 50 per cent hemolysis in the test dose of 0.1 ml.

8. Human Serum, sera received for assay were stored in Pyrex test tubes fitted with paraffin-coated corks. In most instances, because of the presence of visible particles, sera were Seitz-filtered prior to storage at –20° C. For use in test, aliquots were thawed, thoroughly mixed, and heated at 60° C. for ½ hour. Serial twofold dilutions in Veronal buffer were made beginning with 1-4 and ending in 1-128. 0.5 ml. of each dilution was sufficient to perform assays. The test dose was 0.1 ml. of the dilutions.

9. Antigen, whole extract ragweed antigen preserved in an equal volume of glycerin was used. The smallest amount of antigen which fixed the greatest amount of complement (containing four units) in the presence of the smallest amount of rabbit-immune serum was employed in a test dose of 0.1 ml. This amount of antigen contained 0.2 microgram of protein N. No less than 0.1 ml. of stock glycerinized antigen was used in preparing the optimal dilution. Fresh dilutions were made daily. While not in use, the antigen dilution was kept refrigerated.

10. Test Procedure for Inhibiting Antibody, the test procedure followed is outlined in Table IX. The criterion for the presence of inhibiting antibody was the occurrence of 50 per cent or greater hemolysis in test mixtures containing the human serum, antigen, immune rabbit serum, and complement. The results obtained with a given serum were expressed as the highest dilution showing evidence of activity as defined.

Controls included in the assays were:

a. Hemolytic controls with one and two 50 per cent hemolytic C′ units.
b. Antigen controls with one 50 per cent C′ unit.
c. Cell control.
d. Human serum, known to contain inhibiting antibody by previous assay, was tested in a similar fashion as the unknown sera.
e. Human serum, not containing inhibiting antibody, was similarly tested.
f. Immune rabbit serum was tested at the dilution selected and actually used in tests in the presence of four and eight 50 per cent hemolytic units and the

TABLE IX. OUTLINE OF COMPLEMENT FIXATION INHIBITION TEST FOR INHIBITING ANTIBODY AGAINST RAGWEED POLLEN EXTRACT

ORDER OF ADDING REAGENTS	TEST DOSE ML.	TUBE NUMBER 1	2	3	4	5	6	7	8	9	10	11	12
Human serum dilutions	0.1	4	8	16	32	64	128	4	8	16	32	64	128
Antigen	0.1	+	+	+	+	+	+						
Veronal buffer	0.1							+	+	+	+	+	+
Incubate 1 hour 37° C. water bath													
Immune rabbit serum	0.1	+	+	+	+	+	+	+	+	+	+	+	+
Complement, 4 units	0.1	+	+	+	+	+	+						
Complement, 1 unit	0.1							+	+	+	+	+	+
Incubate 4 hours 3-6° C.													
Sensitized cells	0.2	+	+	+	+	+	+	+	+	+	+	+	+
Incubate 15 minutes 37° C. water bath Record degrees of hemolysis													

Human serum dilutions expressed as reciprocal.
+ indicates reagent added.

test dose of antigen employed in the assays on human sera. Also serum controls were set up with this dilution of rabbit antiserum in the presence of one and two 50 per cent C′ units.

Tests were considered satisfactory when 40 to 60 per cent hemolysis was obtained with one unit of C′ in the hemolytic and antigen controls; when inhibiting action was obtained with the known inhibiting serum, and no reaction with noninhibiting serum; when the number of units of C′ required for 50 per cent hemolysis in the presence of the rabbit-immune serum ranged from 8 to 10.

B. *Comparison of Results Obtained in the Assay of Inhibiting Antibody by the Complement Fixation Inhibition Test and the Passive Transfer Test for Blocking Antibody.—*

Tests were made for inhibiting antibody using the complement fixation method as described. Blood specimens were obtained one to four weeks after the previous injection. Independent tests for blocking antibody by passive transfer were performed by a modification of the Loveless method.[7] Sera to be tested were heated 4 hours at 56° C. to inactivate skin-sensitizing antibody. 0.1 ml. of the serum was mixed with an equal volume of several dilutions of low-ragweed extract and left overnight in the refrigerator. Then 0.1 ml. of an unheated serum containing sensitizing antibody was added. 0.1 ml. of these mixtures was injected into nonallergic skin. The sites were tested 48 hours later with approximately 0.02 ml. of low-ragweed extract containing 1,000 protein N. units per milliliter. The sensitizing sera were from untreated ragweed cases and chosen on the basis of neutralization by 50 to 100 units of ragweed extract. Thus if the serum being tested did not contain blocking antibody, the mixture made with 50 units gave a positive reaction, while that with 100 units did not. The titers recorded represent the strength of ragweed extract in the last site giving a positive reaction. The results obtained with the complement fixation and passive transfer methods are presented together with other pertinent data in Table X. With sera in Categories 1 and 2, representing patients not allergic to ragweed, no evidence of blocking antibody by passive

transfer was obtained. Likewise, negative results were obtained in the complement fixation inhibition test. Moreover, 50 sera selected at random from sera giving negative results in serologic tests for syphilis gave no complement fixation findings for inhibiting antibody. No passive transfer tests were made with these sera. Category 3 represents those ragweed-sensitive individuals who failed to complete treatment or to return for bleeding after treatment. Samples tested represent pretreatment bloods. The passive transfer method and complement fixation inhibition test were negative with all sera. Patients in Category 4, subgroup 1, ragweed sensitive and in their first year of treatment, showed evidence of blocking antibody by passive transfer on posttreatment samples. The titers of blocking antibody ranged from 100 to 200. Tests for inhibiting antibody using the complement fixation test were negative with these sera. Patients in Category 4, subgroup 2, ragweed sensitive and in their first year of treatment, showed evidence of blocking antibody by passive transfer on posttreatment samples. (No pretreatment specimens were taken.) In this group, the passive transfer method gave titers of 100 to 500. Evidence of inhibiting antibody in the complement fixation test was obtained with serum 5,077, which gave a titer of 500 in the passive transfer test. In Category 4, subgroup 3, the patients were treated for several years with ragweed extract. The highest titers of blocking antibody, i.e., 200 to 2,000, were obtained in this group. Likewise the highest incidence of inhibiting antibody action in the complement fixation test was obtained with this group. All sera giving titers of 500 or more in the passive transfer test were positive in the complement fixation test. Category 5 contained nonsensitive individuals who were injected with ragweed extract to simulate the formation of blocking antibody. Titers of blocking antibody by passive transfer ranged from 100 to 1,000. Evidence of inhibiting antibody in the complement fixation test was obtained with all samples showing 500 or more units in the passive transfer test.

C. *Reproducibility of the Complement Fixation Inhibition Test for Inhibiting Antibody Against Ragweed Extract.*—

To obtain information relative to the reproducibility of the complement fixation test for inhibiting antibody, tests were made on 46 individual sera on Nov. 1, 1952, and the test repeated using the same lot of antigen and immune-rabbit serum on March 10, 1953. The results obtained are presented in Table XI. Except for two sera, 5,046 and 5,058, the results were reproducible. Sera 5,046 and 5,048 were positive in the original testing, and negative on retesting. However, the degree of hemolysis obtained with both sera in the 1-4 dilution almost placed them in the positive category.

DISCUSSION

In studying the effect of various factors on the detection of antibody against ragweed antigen using the complement fixation inhibition test, the possibility that the observed inhibition might be due to the presence of thermostable complement components was ruled out by parallel and independent studies of the activating effect of the human sera on complement. However,

TABLE X. COMPARISON OF RESULTS OBTAINED IN THE ASSAY OF INHIBITING ANTIBODY BY THE COMPLEMENT FIXATION INHIBITION TEST AND THE PASSIVE TRANSFER TEST FOR BLOCKING ANTIBODY

Category 1—Control Sera

A. PATIENTS NOT ALLERGIC TO RAGWEED AND NOT TREATED WITH ALLERGENS

LAB. NO.	PAT.	SPEC. TAKEN	PASS. TRANS.	C-F INHIB.	CLINICAL DIAGNOSIS
*5060	WC	8-25-52	–	–	Contact dermatitis
5014	JL	3-16-51	–	–	Migraine
*5003	RM	10-10-52	–	–	Ménière's disease
*5084	JM	10-29-52	–	–	Infective asthma
*5009	HM	10-26-50	–	–	Urticaria
*5008	CW	10-26-50	–	–	Monilia dermatitis
*5083	KM	9-13-52	–	–	Infective asthma
5071	WC	6-17-52	–	–	No disease found
*5080	JC	9-24-52	–	–	No disease found
50 sera tested at random, negative in serologic tests for syphilis			Not done	–	Not known

Category 2—Control Sera

B. PATIENTS NOT ALLERGIC TO RAGWEED BUT ALLERGIC TO OTHER POLLENS; SERA TAKEN AFTER TREATMENT WITH OTHER POLLENS

5068	PC	?	–	–	Grass pollens
*5081	CO	12-9-52	–	–	Grass pollens
*5082	KB	12-5-52	–	–	Grass and tree pollens

Category 3—Ragweed Sensitive

A. PATIENTS WHO FAILED TO COMPLETE TREATMENT OR TO RETURN AFTER TREATMENT; SAMPLES TESTED REPRESENT PRETREATMENT BLOODS

5013	CA	3- 2-51	–	–
5015	MO	12-16-51	–	–
5035	MG	3-10-52	–	–
5023	AS	9-10-51	–	–
5036	JD	3-21-52	–	–
*5066	GW	8-25-52	–	–
*5011	RR	11- 6-50	–	–
*5012	SW	11-29-50	–	–

Category 4—Ragweed Sensitive

B. PATIENTS TREATED WITH RAGWEED EXTRACT

1. PATIENTS IN FIRST YEAR OF TREATMENT FROM WHOM BLOOD WAS OBTAINED BEFORE TREATMENT

LAB. NO.	PAT.	SPEC. TAKEN	NO. OF INJECT.	TOP DOSE P.N. UNITS	PASS. TRANS.	C-F INHIB.	CLINICAL RESULT
5034	HM	3-14-52	0	0	–	–	
*5063		6-19-52	13	1200	100	–	
*5062		8-19-52	18	5500	200	–	Excellent
5032	MC	3- 4-52	0	0	–	–	
5049		6-18-52	15	2000	100	–	
5072		8-19-52	23	5000	200	–	Good-excellent
5027	MG	3- 6-52	0	0	–	–	
5051		6-18-52	14	1500	100	–	
5070		8-20-52	23	5000	200	–	Undetermined
5044	RL	5-20-52	0	0	–	–	
5073		8-12-52	12	2000	100	–	Good
5057	LD	4-19-52	0	0	–	–	
5045		6-18-52	9	800	–	–	
5069		8-19-52	17	5000	200	–	Good
5058	ES	4-25-52	0	0	–	–	
5047		6-18-52	8	600	–	–	
5074		8-22-52	16	5000	200	–	Excellent
5075		(duplicate specimen to 5074)				–	

TABLE X—CONT'D

2. PATIENTS IN FIRST YEAR OF TREATMENT FROM WHOM BLOOD WAS NOT OBTAINED BEFORE TREATMENT

LAB. NO.	PAT.	SPEC. TAKEN	NO. OF INJECT.	TOP DOSE P.N. UNITS	PASS. TRANS.	C-F INHIB.	CLINICAL RESULT
*5064	HL	8-13-52	22	11000	100	–	Excellent
*5065	DR	8-22-52	23	6000	200	–	Good
*5077	FB	8-28-52	29	4500	500	Pos. 1:8	Good
*5078	EM	9-22-52	13	8500	100	–	Excellent
*5079	SM	9-13-52	14	2250	200	–	Fair

3. PATIENTS TREATED FOR SEVERAL YEARS; MOST HAD 25 OR MORE INJECTIONS THE FIRST YEAR AND 16 TO 20 IN SUBSEQUENT YEARS

*5001	RL	10- 9-50		10000	200	–	Good
*5005	EC	10-12-50		6000	200	–	Good
*5006	SC	10-12-50			200	–	
5024	JS	10-13-51		35000	1000	Pos. 1:8	Excellent
5025	SR	9-19-51		50000	1000	Pos. 1:8	Excellent
5026	ER	6- 8-51		40000	1000	Pos. 1:16	Good
5067	ER	8-19-52		50000	2000	Pos. 1:32	Good
*5061	MW	8-12-52		17000	500	Pos. 1:8	Good

Category 5—Nonsensitive

PATIENTS GIVEN INJECTIONS OF RAGWEED TO STIMULATE THE PRODUCTION OF BLOCKING ANTIBODY

							DIAGNOSIS
5016	W	12-19-50	0	0	–	–	Vasomotor rhinitis
5017		1-15-51	5	15000	100	–	
5018		3-29-51	10	50000	200	–	
5019		5-15-51	15	50000	1000	Pos. 1:4	
5020	BL	1-15-51	0	0	–	–	Vasomotor rhinitis
5021		5-14-51	10	50000	200	–	
5029	P	10-26-51	0	0	–	–	Vasomotor rhinitis
5028		2- 1-52	12	50000	–	–	
5033		3-14-52	17	50000	100	–	
5048		5- 6-52	24	100000	500	Pos. 1:4	
5030	A	11-19-51	0	0	–	–	Infective asthma
5031		12-28-51	4	34000	–	–	Discontinued inject.
5037	MZ	10-10-51	0	0	–	–	Infective asthma
5038		3- 6-52	8	50000	100	–	
5046		5-28-52	12	50000	500	Pos. 1:4	
5053	RS	11-13-51	0	0	–	–	Infective asthma
5054		4-25-52	17	10000	100	–	
5050		6- 2-52	22	10000	100	–	
5055	RH	11-13-51	0	0	–	–	Infective asthma
5056		4-25-52	10	50000	100	–	
5039	T	10-29-51	0	0	–	–	Chronic sinusitis
5040		3-11-52	11	50000	100	–	
5059		4-18-52	16	70000	200	–	
5052†		6-23-52	25	100000	500	Pos. 1:8	
5043‡		6-26-52	25		500	Pos. 1:8	
5076§		8- 4-52	25		500	Pos. 1:8	

*Sera not Seitz-filtered.
†Blood sample collected 1 hour after the twenty-fifth injection.
‡Blood sample collected 3 days after the twenty-fifth injection.
§Blood sample collected 5 weeks after the twenty-fifth injection.

TABLE XI. REPRODUCIBILITY OF THE COMPLEMENT FIXATION INHIBITION TEST FOR HUMAN ANTIBODY AGAINST RAGWEED ANTIGEN

LAB. NO.	COMP. FIXATION RESULTS ORIGINAL	RETEST	LAB. NO.	COMP. FIXATION RESULTS ORIGINAL	RETEST
5021	Neg.	Neg.	5053	Neg.	Neg.
5024	Pos. 1:4	Pos. 1:8	5054	Neg.	Neg.
5025	Pos. 1:8	Pos. 1:8	5056	Neg.	Neg.
5027	Neg.	Neg.	5057	Neg.	Neg.
5029	Neg.	Neg.	5058	Neg.	Neg.
5030	Neg.	Neg.	5059	Neg.	Neg.
5031	Neg.	Neg.	5060	Neg.	Neg.
5033	Neg.	Neg.	5061	Pos. 1:8	Pos. 1:8
5034	Neg.	Neg.	5062	Neg.	Neg.
5035	Neg.	Neg.	5063	Neg.	Neg.
5037	Neg.	Neg.	5064	Neg.	Neg.
5038	Neg.	Neg.	5065	Neg.	Neg.
5039	Neg.	Neg.	5066	Neg.	Neg.
5040	Neg.	Neg.	5067	Pos. 1:32	Pos. 1:32
5043	Pos. 1:4	Pos. 1:8	5068	Neg.	Neg.
5044	Neg.	Neg.	5069	Neg.	Neg.
5045	Neg.	Neg.	5070	Neg.	Neg.
5046	Pos. 1:4	Neg.	5071	Neg.	Neg.
5047	Neg.	Neg.	5072	Neg.	Neg.
5048	Pos. 1:4	Neg.	5073	Neg.	Neg.
5049	Neg.	Neg.	5074	Neg.	Neg.
5050	Neg.	Neg.	5075	Neg.	Neg.
5052	Pos. 1:8	Pos. 1:8	5076	Pos. 1:4	Pos. 1:8

Original tests done on Nov. 1, 1952.
Retests done on March 10, 1953.

since the bulk of the experiments made were with aged sera, it is conceivable that tests with fresh sera may occasionally produce an inhibitory type reaction due to thermostable complement components. For this reason the parallel use of serum controls, in the presence of an amount of complement which will normally produce partial hemolysis, i.e., one 50 per cent hemolytic unit, would be desirable in performing assays for inhibiting antibody.

Studies on the effect of the time and temperature of preliminary incubation of antigen and human serum prior to the addition of the complement fixing rabbit-immune serum and complement (Tables I and II), indicated that a relatively short exposure of one hour was sufficient. Moreover, tests without preliminary incubation showed some evidence of inhibition but to a lesser degree than with preliminary incubation of antigen and human sera. No greater degree of inhibition was observed when complement was present during the preliminary incubation period. Slightly more sensitive findings were obtained with a preliminary incubation at 37° C. than at 5° C. for similar periods of time. It seemed conceivable that this greater sensitivity at 37° C. might have been due to nonspecific deterioration of the antigen at that temperature. It was noted, however, that as little as 0.002 ml. of serum prevented the deterioration of antigen at this temperature (unpublished findings). Since the smallest amount of serum tested for inhibiting action was approximately 0.007 ml. it would seem that the greater sensitivity at 37° C. was probably not due to deterioration of antigen. Conceivably, inhibiting antibody has a greater combining power for antigen at the higher temperature.

Previously, Hilleman and associates[49] studied inhibitory antibodies present in chickens immunized against ornithosis virus. They noted that the end points of reactivity were unchanged when the preliminary exposure of inhibiting serum and antigen was varied from 30 to 120 minutes. Moreover, it was noted[49] that the presence of complement during the preliminary incubation period of inhibiting antibody and antigen did not increase the reactivity. The findings obtained in the present study with respect to these factors were similar.

Previous investigations indicated that the order of mixing the antisera affected the results of inhibition test. Thus Goodner and Horsfall[47] studied antipneumococcal horse (noncomplement-fixing) and rabbit (complement-fixing) antisera in relation to the fixation of complement in the presence of homologous polysaccharide. They observed that the serum added last, prior to the addition of complement, especially if in excess, conferred its properties on the antigen-antibody complex. Rice[41] obtained similar findings using the same system. However, she stressed the importance of the antigen dose, noting that if an excess of nonfixing horse antiserum was incubated with less than its neutralizing dose of antigen, prior to the addition of complement-fixing rabbit antiserum, almost complete inhibition occurred upon subsequent addition of rabbit antiserum and complement. When rabbit serum was added first under the same conditions, subsequent addition of horse serum and complement did not cause inhibition. When a small excess of horse antiserum was added after an interval to a mixture of rabbit antiserum, antigen, and complement, the added serum apparently checked further fixation by the rabbit antiserum but did not have a reversing effect on the fixation which had already occurred. Hilleman and associates[49] noted that when the fixing serum was allowed to react with antigen and complement, the subsequent addition of inhibiting children serum did not inhibit the fixation. In addition, if inhibiting serum, human serum, complement, and antigen were all mixed together, inhibition occurred but to a lesser degree. In the present studies it was also noted (Table II) that some inhibition occurred when ragweed-inhibiting human serum, complement-fixing rabbit serum, antigen, and complement were allowed to react without preliminary exposure of antigen to inhibiting serum. Whether the two antibodies are competing for the same antigen groupings cannot be determined in view of the lack of purity of the ragweed antigen and probable heterogeneity of the antibodies. Highly purified ragweed antigens and potent specific antisera, both rabbit and human, would provide the means for an approach to this problem.

The importance of the antigen dose to the degree of inhibition observed was noted by Calmette and Massol,[44] Hilleman and associates,[49] Wolfe and associates,[48] Karrer and associates,[50] and Rice and Gwatkin.[41] In most instances the selection of the antigen dose was empirically made to be compatible with a high sensitivity.[44, 48-50] Rice[43] recommended the use of an antigen dilution which gave maximal reaction with a "standard serum" having a complement-fixing capacity of six to eight units on the basis of theoretical and experimental consideration.[41]

In the studies reported herein, the selection of the antigen dose at 0.2 microgram of protein N (whole extract) was made empirically. However, this dosage of antigen also represented the optimal dilution for obtaining maximal reactions with the smallest amount of rabbit-immune serum. From Table IV it was noted that the greatest degree of inhibiting activity occurred with 0.2 microgram of antigen protein N. When twice this amount of antigen, 0.4 microgram, was used the reactivity was slightly reduced. When larger amounts of antigen were used, as, for example 0.8 microgram (Figs. 1 and 2), the results were difficult to interpret. Since 0.8 microgram of antigen protein N. was in excess for the amount of rabbit-immune serum as seen by the reduced fixation of C′, partial neutralization of antigen by inhibiting antibody should have produced a fixation of C′ possibly obtained with 0.2 or 0.4 microgram of antigen. It is conceivable that the neutralization of antigen by inhibiting antibody may be subject to the effects of antigen excess as is the interaction of antigen with complement-fixing rabbit antibody.

The most important factor in maintaining the specificity of the inhibition reaction appeared to be the concentration of the complement-fixing rabbit antiserum. The importance of the dosage of fixing serum was shown by Hilleman and associates,[49] and a recommendation was made by Rice,[43] on the basis of theoretical and experimental considerations, that the fixing serum be employed in a dilution which fixes six to eight 50 per cent hemolytic units of C′. In the present work it was found that a dilution of rabbit antiserum which required eight to ten units of C′ for 50 per cent hemolysis gave specific findings (Table VIII). Smaller amounts of rabbit antiserum produced nonspecific findings. The inhibition occurring nonspecifically when smaller amounts of rabbit antiserum were employed may be more apparent than real. In performing comparative titrations of serum in tests for syphilis, it has been noted that higher titers are obtainable when serum is diluted in saline than when diluted in normal serum. Reduction in the amount of complement fixed by the rabbit-immune serum in the presence of human sera may be related to a similar effect especially when the concentration of rabbit serum used is low.

The importance of the dosage of fixing antiserum used in the detection of inhibiting antibody is further stressed by the experiment performed in these studies on the effect of varying the incubation period following the addition of rabbit-immune serum. Hilleman and associates[49] noted reduced inhibitory action for chicken antisera when the incubation period following the addition of human-fixing serum was prolonged, but did not offer any explanation for this phenomenon. From the studies reported here, the decrease in inhibiting powers of ragweed-inhibiting serum with increasing time of secondary incubation appeared to be related to the increasing fixation of rabbit antiserum with time (Table VII). Obviously, the selection of the optimal dose of fixing antiserum should be determined by the complement-fixing power of the serum for the period of time selected for the test. Decreased reactivity of inhibiting serum might be anticipated if, for any reason, the period of fixation was prolonged.

The decision to use a fixation period of 4 hours rather than 18 hours was based on the results given in Table VII. Although the shorter period of 4 hours is inadequate for maximum fixation of rabbit antiserum, the results obtained in tests for inhibiting antibody indicated that there was a greater difference in the behavior of serum containing inhibiting antibody from normal serum using the shorter (4-hour) incubation period.

Studies made on the effect of time and temperature of heating of the inhibiting antibody (Table V) reflected the thermostability of this antibody. Since no marked differences were obtained with an aliquot heated at 56° C. for ½ hour from an aliquot heated at this temperature for 4 hours, it did not seem that sensitizing antibody, likewise originally present in serum 5026, exerted any effect on the observed reactivity of the inhibiting antibody. (Sensitizing antibody is supposed to be destroyed by heating for 4 hours at 56° C.)[7] The selection of 60° C. for ½ hour as the heating temperature and time for assay of inhibiting antibody was made since equivalent reactivity was obtained with aliquots heated at this temperature and at 56° C. for 4 hours, since Loveless[7] had shown that this time and temperature were equally as effective as 56° C. for 4 hours in assays of blocking antibody by the passive transfer test, and since the shortened time of exposure was more convenient for performing the tests.

In the study of the comparative reactivity of inhibiting human serum against different antigens, essentially similar degrees of reactivity were obtained with whole extract antigen, "Fraction 1," "Fraction 1P," and Fraction 1S." (Table VI.) Since rabbit sera of sufficient titer were not available to make tests for inhibiting antibodies against "Fractions 2 and 3," no conclusions can be drawn about the behavior of these antigens in tests for inhibiting antibodies using this method. It is pertinent to recall that Sherman and Stull[55] had noted that "Fraction 1" was allergenically more reactive than the other fractions in skin tests using sera from ragweed-sensitive cases.

In the method ultimately selected for use in the assay of inhibiting antibody, the decision was made to use four 50 per cent hemolytic units in the test because of the anticomplementary properties of the sera submitted for assay. It seemed possible that with less complement such anticomplementary action might mask an inhibitory effect. Whenever possible the use of fresh sera or sera stored at –20° C. soon after blood collection would be desirable. Moreover as can be seen from Table IV, the use of larger amounts of C′, i.e., six and eight units produced nonspecific findings. Obviously, the amount of complement used should be considered in relation to the antibody content of the fixing-serum dilution used.

In this study, 46 individuals contributed 77 sera on which the passive transfer and indirect complement fixation inhibition tests were made. It was found that in all instances where a titer of 500 or higher was obtained with the passive transfer method, positive reactions were obtained with the complement fixation inhibition test. Seven sera gave titers of 500 with the passive transfer method. Of these, two gave 1-4 reactions in the complement fixation inhibition

test, while five gave 1-8 reactions. Four sera gave titers of 1,000 in the passive transfer test. Of these one gave a 1-4 reaction; two, a 1-8 reaction; and one, a 1-16 reaction in the complement fixation inhibition test. Only one serum gave a titer of 2,000 in the passive transfer test, and this gave the highest titer, 1-32, in the complement fixation inhibition test. These results suggest a correlation between the passive transfer test for blocking antibody and the complement fixation test for inhibiting antibody. It is possible that the complement fixation test is measuring the same antibody as is measured in the passive transfer test. The specificity of the complement fixation test is attested by the failure to obtain positive reactions in those instances where the passive transfer method gave negative findings. Moreover, 50 sera selected at random from specimens giving negative tests for syphilis likewise gave negative results in the complement fixation test for inhibiting antibody. The high specificity of the complement fixation inhibition test is undoubtedly correlated with the lower sensitivity of this test in comparison to the passive transfer method.

Another possible explanation for the observed inhibition with the sera of ragweed-treated cases was the presence of a nonspecific factor which interfered with the fixation of complement. In order to obtain additional proof for the specificity of the complement fixation inhibition test for ragweed antibodies, several sera, containing inhibiting antibodies for ragweed antigen, were tested for their effect on the fixation of complement by an unrelated antigen-antibody system. By using the identical test conditions described in this report for the assay of inhibiting antibodies against ragweed antigen, sera 5,025, 5,061, and 5,067, which contained inhibiting antibodies for ragweed antigen, were tested for their effect on the fixation of complement by a standardized, recrystallized egg albumin-antiegg albumin system. The sera tested produced no discernible effect on the fixation of complement by this unrelated system.

In considering the duration of the injection treatments to the development of blocking and inhibiting (cf) antibodies it can be seen from Table X, that the highest blocking antibody titers as well as the highest incidence of positive reactions in the complement fixation test were obtained in the ragweed-sensitive group treated over several years.

On the basis of the results obtained, no conclusions could be drawn as to the relationship of blocking or inhibiting (cf) antibodies to clinical benefits. This is a controversy that has not been settled by considerably larger series of cases. However, the complement fixation inhibition method may offer a convenient tool for future large-scale studies of the occurrence of this type of antibody as well as provide the allergist with an in vitro method for possibly measuring adequacy of desensitizing treatment. In view of the lower sensitivity of the complement fixation inhibition test and its correlation with the passive transfer method, the complement fixation test could be used as a screening procedure. Those sera, positive in the complement fixation inhibition test, would not have to be tested with the passive transfer technique. Moreover, blood samples collected from selected cases over periods of time could be

stored and tests made on all samples on a given testing day, eliminating many factors of variation inherent in the passive transfer test.

Malkiel and Feinberg[68] studied the production of blocking antibody in thirty-six normal individuals using eight different types of ragweed extracts and could not find any significant increase of blocking antibody. In the present study, eight nonsensitive individuals were injected with ragweed extract. Blocking antibody was produced in seven of the eight cases. The patient who did not develop demonstrable antibody received only four injections. Blocking antibody titers of 200 or more were obtained with five of the cases. In explanation of the discrepancy in the findings of Malkiel and those of the present study it should be noted that Malkiel injected dosages into the normal donors comparable to those given to the ragweed-sensitive cases in the present study. Five to ten times the amounts used in treating the ragweed-sensitive cases were used for injecting the normal donors in the present study. This stresses the importance of adequate dosage in producing blocking antibodies. Inhibiting antibody activity in the complement fixation test was obtained in four of the eight cases, in those cases where titers in the passive transfer method were 500 or greater. With patient T. (Table X, Category 5) it can be noted that there was no change in the titer by passive transfer or in the complement fixation reaction in samples obtained 1 hour, 3 days, and 5 weeks after the twenty-fifth injection. This study was included in order to determine whether the time of obtaining a blood sample after injection would influence the antibody assay. However, no conclusions can be drawn from results obtained with one individual.

SUMMARY AND CONCLUSIONS

1. A study of various factors influencing the determination of human antibodies against ragweed antigen was made using the complement fixation inhibition procedure.

2. As a result of these studies a complement fixation inhibition test was developed and is presented.

3. Comparative studies of the complement fixation inhibition test and the passive transfer method revealed that the former was less sensitive but was correlated with the passive transfer test. Sera giving a reaction of 500 units or more in the passive transfer test were positive with the complement fixation inhibition test.

4. It is suggested that the complement fixation inhibition test herein described may be of value as a screening procedure to determine the development of antibodies in ragweed-treated cases, and in other studies of the immunology of pollinosis. Further, this method may find application to the study of other allergens and their respective antibodies.

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COLLECTIVE ASTHMA, SIMULATING AN EPIDEMIC, PROVOKED BY CASTOR-BEAN DUST

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ON Aug. 11, 1952, the Public Health Department of the State of São Paulo was notified that in Baurú, a town of 60,000 inhabitants, in São Paulo, Brazil, there had appeared an odd type of asthma, apparently of an epidemic nature inasmuch as in a few days 150 cases were registered, with 9 deaths attributed to that illness. The symptomatology consisted of extremely violent dyspena of the asthmatic type which started suddenly. On account of its apparently epidemic character and seriousness from a clinical viewpoint, the illness was at first attributed to a pneumotropic virus. Subsequent investigation showed that these were cases of bronchial asthma provoked by castor-bean dust. The origin of this allergen was found in a large castor-bean crushing mill which exists in the town of Baurú.

The term "epidemic asthma" has been improperly used to designate the collective asthmogenic action of an allergen in the form of an epidemic. Ancona, in 1923, published a paper on "epidemic asthma" brought about by a cereal parasite: *Pediculoides ventricosus.*

As far as the castor bean *(Ricinus communis)* is concerned, cases of asthma of an epidemic character have been described by Figley and Elrod in 1928 and by Grimm in Germany in 1928. In the 30 cases of asthma described by Figley and Elrod in Toledo, Ohio, it was proved that the allergen was the dust from the castor-bean dust, and in 55 cases there were quite evident indications that the etiology was the same. In Grimm's description 30 cases were studied in which scratch tests made with dust from castor beans gave strongly positive reactions.

MATERIAL, METHODS, AND RESULTS

Thirty patients were studied: 9 in the Hospital das Clínicas of the University of São Paulo, 4 in the town of Baurú, and 17 in Agudos, a small town 15 kilometers from Baurú.

The following methods of etiologic diagnosis were used: (1) cutaneous tests; (2) local passive transfer of antibodies; and (3) experimental provocation of symptomatology.

1. *Cutaneous Tests.*—The selection of extracts for cutaneous tests was made by choosing possibly allergenic substances in the industrial field and in the cereal warehouses of the town of Barurú where there are important oil mills, principally pressing cotton seed, peanut, and castor bean. Twelve extracts for cutaneous reactions were prepared with the following origins: seven

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extracts composed of the principal forms through which cotton seed and linters go; three kinds of dust from cereals; one sample from castor-bean dust which is known as castor-bean pomace.

The extracts were prepared with coca solution, dialyzed, filtered through the Seitz filter, and diluted 1:10, 1:100 and 1:1,000 for use in intracutaneous tests.

The group of 9 patients brought to the Hospital das Clínicas was tested with all the extracts; at first with a scratch test using extracts diluted 1:10 and 1:100 and thereafter with intracutaneous tests using extracts diluted 1:1,000. All the patients responded with strongly positive scratch tests to the castor-bean pomace extracts. Four of these patients also presented positive intracutaneous tests with extracts of cotton seed and cereal dust. These patients had been suffering from rhinitis and asthma for several years.

The other 21 patients observed in Baurú and Agudos were tested exclusively by scratch test with the use of only three extracts: castor-bean pomace diluted 1:100; a mixture of cotton seed dusts at 1:10 and a mixture of cereal dusts. Of these 21 patients, 19 showed cutaneous reactions strongly positive to castor-bean extract. Four patients, asthmatic for a long time, also presented positive cutaneous reactions to other inhalants.

On two patients the scratch test with castor bean was negative. One of these patients was an old asthmatic individual, but the other patient was probably a misdiagnosed case for she complained only of shortness of breath and showed no other symptoms of asthma.

2. *Local Passive Transfer of Antibodies.*—At the Hospital das Clínicas, the local passive transfer of reagins was made with the sera of 9 patients. The patients' sera were filtered through the Seitz filter and a volume of 0.05 ml. was injected intracutaneously in the dorsal region of two nonallergic patients. Twenty-four hours later 0.03 ml. of castor-bean extract diluted 1:1,000 was injected in the same site, previously prepared with the serum. Comparative test injections were made only with the extract.

The sera of all of the 9 patients provoked strongly positive Prausnitz-Küstner reactions.

3. *Experimental Reproduction of Symptomatology.*—Four of the patients admitted to the Hospital das Clínicas were selected to be submitted to experimental asthma provoked by the inhalation of extract of castor-bean pomace. Two of these patients were already suffering from asthma before the epidemic; one already had allergic rhinitis and the other had already suffered from asthma or allergic rhinitis up to the time the collective type of asthma broke out.

As the allergen was used, an extract was made in the usual way, in coca solution, dialyzed and filtered, with 1,240 gammas of protein nitrogen per milliliter.

In a 36-cubic-meter carefully calked room were placed four No. 40 DeVilbiss nebulizers, one at each corner and 2 meters high. The four nebu-

lizers were connected with rubber tubes to the air injector of a vacuum pump. In each of the four nebulizers were placed 3 ml. of extract of castor-bean pomace. The nebulizers were operated for an hour.

The four patients were previously examined and did not show either symptoms or signs of asthma during a period of over 15 days. In order to remove psychological factors, the patients were not informed of the experiment and were directed to the room on the excuse of a radiologic examination.

The following were the results: after one minute had elapsed one of the patients already showed allergic nasal and eye symptoms and after two minutes she was withdrawn from the room and had developed a typical attack of asthma. The other three patients began to show symptoms after three minutes characterized by sneezes, pruritus of the eyelids, cough, and accentuated dyspnea which called for their withdrawal from the room.

TABLE I. PRINCIPAL DATA GATHERED FROM OBSERVATIONS

CASE	SEX	AGE	P.A.H.	F.A.A.	C.R. TO CASTOR BEAN	C.R. TO OTHER ALLERGENS	P.K.	EXPERIMENTAL ASTHMA
1	F.	25	Asthma and rhinitis	–	+	+	+	+
2	F.	63	—	–	+	–	+	+
3	F.	24	Asthma	–	+	–	+	+
4	F.	20	Rhinitis	–	+	+	+	+
5	M.	33	Rhinitis	+	+	+	+	
6	M.	20	—	–	+	–	+	
7	M.	70	—	–	+	–	+	
8	M.	18	Rhinitis	+	+	–	+	
9	M.	17	Asthma and rhinitis	–	+	+	+	
10	M.	36	Asthma	+	+	–		
11	M.	9	Asthma and rhinitis	+	+	+		
12	F.	8	—	–	+	+		
13	F.	28	Rhinitis	–	+	–		
14	F.	25	Rhinitis	–	+	–		
15	F.	38	—	–	–	–		
16	M.	20	Asthma and rhinitis	+	+	+		
17	F.	35	Asthma	+	+	–		
18	M.	43	Asthma	+	+	+		
19	M.	12	—	+	+	–		
20	M.	34	—	–	+	–		
21	M.	9	—	–	+	–		
22	F.	54	Asthma	–	+	–		
23	M.	54	Asthma	–	+	+		
24	M.	20	—	–	+	–		
25	M.	39	Asthma	+	–	+		
26	F.	45	—	–	+	–		
27	M.	30	Asthma	–	+	–		
28	M.	3	Asthma	+	+	+		
29	M.	32	—	–	+	–		
30	M.	38	—	–	+	–		

P.A.H. = Personal allergic history.
F.A.A. = Familial allergic antecedents.
C.R. = Cutaneous reaction.
P.K. = Prausnitz-Küstner reaction.

The four patients had serious attacks of bronchial asthma which, in spite of the administration of epinephrine, aminophylline, cortisone and oxygen, lasted about two hours. At the end of the experiment the four patients affirmed that the symptoms had been identical with the symptoms observed in

the town of Baurú. Three physicians and two nurses who took part in the experiment and were submitted therefore to the inhalation of castor-bean extract did not show any symptoms.

DATA OBTAINED FROM HISTORY

Sex.—There were twelve female and eighteen male patients.

Age.—The youngest patient was three years, and the oldest was seventy years old. The following are the age groups of our patients.

0-10 years	11-20 years	21-30 years	31-40 years	41-50 years	51-60 years	61-70 years
6 cases	4 cases	6 cases	8 cases	2 cases	1 case	2 cases

Allergic History.—The history disclosed the following:

a. 12 patients already suffered from asthma before Aug. 11.

b. 6 patients suffered previously from allergic rhinitis.

c. 16 patients had asthma for the first time between Aug. 11 and 16.

d. 7 patients showed hereditary allergic histories.

e. In 10 patients the asthmatic symptoms were followed and sometimes preceded by rhinitis, conjunctivitis, and pruritus of the ears, nose, palate, and eyelids.

f. The majority of the patients rid themselves of the symptoms when they left the town of Baurú, for which reason 17 of the sick transferred themselves to the town of Agudos, 15 kilometers away. In this latter town the patients exclusively allergic to castor bean did not suffer any symptoms.

g. The symptoms appeared again when the patients returned to Baurú. There were patients who made from four to five attempts to return, but every time they did so they had violent attacks of asthma.

h. According to observations made by some patients, whenever it rained the symptoms disappeared or decreased in intensity. The direction of the wind also influenced it, in the opinion of the nurses and physicians of the Hospital of Baurú.

i. The symptoms disappeared in all patients allergic solely to castor bean when the crushing mill ceased to work.

j. A new episode, again of an epidemic nature, took place one month after the crushing mill resumed work.

DISCUSSION

The clinical data along with the cutaneous tests, the passive transfer of antibodies and the experimental reproduction of the symptomatology were sufficiently suggestive to prove we were dealing with patients who had allergic manifestations of the respiratory tract, with predominating asthmatic symptoms whose etiologic agent was the dust from the castor bean. As a matter of fact some patients (Cases 12 and 20) made three attempts to return to the town of Baurú and every time they had attacks of asthma which compelled them to return to the town of Agudos, situated at a distance of 15 kilometers. The direction of the wind and rain seemed to interfere, since some patients in the local hospital showed no symptoms on rainy days while

others became worse when the wind blew in a certain direction. This observation had already been made by Grimm in Germany, inasmuch as according to the direction of the wind the attacks took place in certain districts.

With reference to the allergen of castor-bean dust, the following points should be stressed:

1. The great capacity of sensibilization is noted, a fact already known by other authors such as Ratner with experiments on animals. The allergen is extremely potent and sensibilizes with certain facility, since in our observations 38.5 per cent of the patients had no previous allergic manifestations.

2. The allergenic source being found in the bean dust and not in the oil explains the manner in which the allergen spread itself in the atmosphere of Baurú. In that locality there is a large castor oil mill which has been operating for several years. In order to secure a better yield of oil, at the time of the epidemic outbreak the mill started a new process of extraction which consists of treating castor-bean pomace with hexane. After this treatment the cake is thrown into the grinder and thence into a "cyclone" preparatory to sacking the castor-bean pomace. During the period of sacking the ground castor-bean pomace an atmosphere loaded with dust from castor-bean residues is formed. Through ventilators this powder is in a large part transferred to the outside of the mill. This new method has probably contributed to render the allergen more diffused in the atmosphere and under more suitable conditions to sensibilize. In this way it can be explained that the cases of collective asthma of an epidemic nature occurred one day after the changes made in the method of extracting castor oil.

3. The active allergenic principle studied by Barnard is soluble in water and precipitated by alcohol and is not destroyed by heat. Based on these properties of the allergen, the mill changed the method of extraction by causing the castor-bean dust to pass through water receptacles in order to retain the allergen and keep it from concentrating in the atmosphere. With this new method the mill resumed work two months ago after having been inactive for one year.

4. Two patients who had not been to Baurú for a long time had attacks of asthma a few hours after their arrival. One of them (Case 24) had not been to Baurú for six months and upon arriving in that town had an attack of asthma. This patient worked with fertilizers, including castor bean, in the town where he lived and must have thus become allergic to it. Allergy from castor-bean fertilizers is well known, and Vaughan published three cases.

Some patients continued to have asthma in spite of leaving Baurú (Case 16), owing to their being old asthmatic persons with other sensitizations. These patients, allergic to various substances, continued to have their usual attacks even during the time the mill was closed, but not with the severity observed during the so-called epidemic period.

The patients suffering from asthma exclusively from castor-bean pomace had no further attacks after the factory was closed and adopted the new protective measures.

Recently, on studying allergy to castor seed fertilizers, Small stated that scientific proof can be obtained by making the patient inhale the allergen under certain conditions, but this was not encouraged for the castor bean is an extremely potent allergen. We succeeded in the experimental reproduction of asthma on human beings provoked by castor bean (Cases 1, 2, 3, and 4) with a method that is close to natural conditions, inasmuch as the nebulization was not made with the apparatus in direct contact with the patient, and the psychic factors of suggestion were removed.

With the greater industrial development, we are convinced that identical episodes will be observed not only with castor bean, but with other potent allergens such as those derived from cotton seed, etc. It is also likely that in the vicinity of the castor oil or cotton seed oil mills there is a greater incidence of allergic manifestations, principally of the respiratory tract, than in the population in general.

SUMMARY

In August, 1952, in Baurú, a city of 60,000 inhabitants, in the State of São Paulo, Brazil, the Public Health Service was notified within a few days of 150 cases of a particular type of bronchial asthma, and nine deaths were attributed to it. The illness simulated an epidemic.

Allergic studies in 30 cases revealed that they were cases of bronchial asthma and other allergic manifestations of the upper respiratory tract brought about by allergy to castor-bean *(Ricinus communis)* dust.

The etiologic proof was made by history; strongly positive cutaneous tests; passive transfer of the skin-sensitizing antibodies, and by experimental provocation of symptoms.

The experimental reproduction of the symptoms was possible by placing 4 patients in a room in which had been nebulized for an hour a 1:10 solution of the allergen. The four patients had extremely severe attacks of asthma, one to three minutes after they went into the room.

The cases of collective asthma coincided with the changes made in the method of extraction of castor-bean oil by hexane, which occurred one day before at the large mill operating for some years in the city of Baurú.

A new outbreak of collective asthma identical with the first one was observed about a month later when the mill resumed work after a period of inactivity and observation demanded by the physicians of the Public Health Service.

With the closing down of the mill for a year no new cases of asthma of a collective nature were observed.

The mill is now working again using a method of catching the dusts in a water reservoir, based on the fact that the allergen is soluble in water and precipitated by alcohol.

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STUDIES IN BACTERIAL ALLERGY*

VI. Hapten Aerosol Desensitization of Guinea Pigs Passively Sensitized to Specific Pneumococcal Polysaccharides

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INVESTIGATION of the many problems of desensitization has been regrettably dormant. A good deal is known about desensitization with whole antigens.[1, 8] Little is known about desensitization with haptens, although anaphylactic shock from bacterial polysaccharides was reported in 1926.[2] Theoretically, desensitization with haptens is of considerable importance, since haptens may neutralize antibodies but do not elicit them primarily or anamnestically. Desensitization with whole antigens is transient.[3] Theoretically, desensitization with haptens should be lasting, in the intact animal, after antibody production has ceased.

Previous studies in this laboratory have shown that desensitization of uterine strips of passively sensitized guinea pigs with pneumococcal polysaccharides was transient when brought about by additions of the hapten to the bath.[4] On the other hand, in intact guinea pigs sensitized passively with heterologous serum, desensitization with the same polysaccharide, intra-abdominally, was apparently as lasting as passive sensitization.[5]

A good deal of attention has been given, recently, to aerosols in a variety of uses. This report deals with some qualitative observations on aerosol desensitization, using a pneumococcal polysaccharide as a hapten, in guinea pigs sensitized passively with specific heterologous antiserum.

METHODS

Guinea pigs were used as experimental animals. Pneumococcal polysaccharide type VI was used and is referred to as a hapten. Type VI refined antipneumococcal rabbit serum was used as the source of heterologous, passively sensitizing antibody.

Passive sensitization was produced, by intra-abdominal injections of 0.35 to 1.0 ml. of antiserum. Occasionally, more was used.

A DeVilbiss No. 40 nebulizer was connected with a source of compressed air, bubbled through water. The hapten was nebulized into a small glass box. This aerosol chamber was not quite airtight. The aerosol fog was kept dense enough to ooze visibly from the loose seams.

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Preliminary aerosol studies with acacia* and with the polysaccharide showed that reactions usually occurred, within two to five minutes, if they were going to. If no reaction occurred, in five to seven minutes, the challenge was considered negative.

Positive reactions were graded *slight dyspnea* when the respiratory volume per breath increased, definitely and unequivocally, usually with some slowing of respiration. *Moderate dyspnea* was used to describe dyspnea which was deep, slow, associated with definite use of the accessory muscles of respiration, and obviously distressing. Dyspnea was called *severe* when it looked as though asphyxia were imminent. *Anaphylactic death (A. D.)* described the severest reactions, in which the animal behaved, objectively, as though he had received a fatal intravenous challenging dose of antigen and had typical ballooned lungs at post mortem.

Slight-moderate and *moderately severe* were used, to describe intermediate degrees of reactions.

Local desensitization was assumed to be present when the guinea pigs were no longer affected by relatively prolonged exposures (ten minutes) to the fog, which, previously, had caused unequivocal dyspnea, promptly.

The first three experiments are not concerned with desensitization. They are presented as background for the desensitization studies.

TABLE I. INTRA-ABDOMINAL TYPE VI ANTIPNEUMOCOCCAL RABBIT SERUM SENSITIZES GUINEA PIGS TO INHALATION OF TYPE VI PNEUMOCOCCAL POLYSACCHARIDE AEROSOL

GP #	I.A.* SENS. DOSE TYPE VI ANTISERUM (ML.)	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	TIME OF EXPOSURE (MIN.)	REACTION†	RECOVERY TIME (MIN.)
1	0.5	3.0	1	A. D.‡	—
2	0.65	3.0	1	Severe	15
3	0.65	3.0	1½	Moderate	10
4	0.65	3.0	1	Severe	10
5	0.65	3.0	6	None	—
6	0.65	1.0	1	A. D.	—
7	0.65	1.0	4	None	—
8	0.65	1.0	1½	Mod.-severe	9
9	0.65	1.0	5	None	—
10	0.65	0.3	1¼	Moderate	10
11	0.5	0.6	4	Slt.-mod.§	4
12	0.5	0.2	4	Moderate	—
13	0.5	3.0	10	None	—
14	0.5	3.0	10	None	—
15	0.5	0.6	8	Slt.-mod.	4
16	0.5	0.2	4	Moderate	—
17	0.5	3.0	7	Slt.-mod.	3
18	0.5	3.0	10	None	—
19	0.35	3.0	1	A. D.	—
20	0.35	3.0	1	Mod.-severe	6
21	0.35	3.0	1	Moderate	6

*I.A.—Intra-abdominal.
†Reactions—Grades of dyspnea; see text.
‡A. D.—Anaphylactic death.
§Slt.-mod.—Slight to moderate dyspnea.

*Acacia was used, here and elsewhere in this report, because its immunologic properties are being studied in this laboratory. Thus, a handy anaphylactic system is provided for comparison. The acacia studies will be reported separately.

Experiment I (Table I).—Guinea pigs are sensitized to inhalation of type-specific polysaccharide aerosol, by intra-abdominal injections of type VI anti-pneumococcal rabbit serum.

Note that there were differences in the time of onset and in the intensity of the reactions and that several animals did not react at all.

Experiment II (Table II).—Illustration of effective thresholds for aerosols of polysaccharide and of histamine. Note that a concentration greater than 0.03 mg./ml. of polysaccharide was necessary, to elicit a reaction. Only one reaction has been elicited by an aerosol concentration of 0.05 mg./ml., so far. There have been several reactions to concentrations of 0.1 and 0.2 mg./ml. Note too that there was a sharp difference in the intensity and in the reaction time between histamine 1:15,000 and 1:3,000.

TABLE II. ILLUSTRATION OF EFFECTIVE THRESHOLDS FOR AEROSOL CONCENTRATIONS OF POLYSACCHARIDE AND OF HISTAMINE

	PASSIVELY SENSITIZED GUINEA PIG				
GP#	I.A.* SENS. DOSE TYPE VI ANTISERUM	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	TIME OF EXPOSURE (MIN.)	REACTION*	RECOVERY TIME (MIN.)
1	0.5 ml.	0.003	8	None	—
		0.03	10	None	—
		0.3	1¼	Moderate	10
2	0.5 ml.	0.001	7	None	—
		0.01	10	None	—
		0.1	6	Slt.-mod.	10
		0.2	4	Mod.-severe	—
	HISTAMINE ALONE				
3	None	1:15,000	6	Slt.-mod.	2
	None	1:3,000	1	Severe	5
4	None	1:15,000	4	Slt.-mod.	1
	None	1:3,000	1	Severe	—

*See legend for Table I.

Experiment III (Table III).—Reactions to polysaccharide haptens differ with the route of administration. Negative reactions to aerosol may be followed by anaphylactic death from intravenous polysaccharides.

TABLE III. REACTIONS TO CHALLENGING DOSAGES OF POLYSACCHARIDE DIFFER WITH ROUTE OF ADMINISTRATION: NEGATIVE REACTIONS TO AEROSOL FOLLOWED BY ANAPHYLACTIC SHOCK FROM INTRAVENOUS POLYSACCHARIDE

GP#	I.A. SENS. DOSE ANTI-SERUM (ML.)	CONC. OF POLYSAC. AEROSOL (MG./ML.)	TIME OF EXPOSURE (MIN.)	REACTION	INTERVAL (HR.)	I.V. POLY-SAC. (MG.)	REACTION
	Type VI	Type VI				Type VI	
1	1.0	4.0	14	0	20	4.0	A. D.
2	0.5	3.0	7	0	1	1.5	A. D.
	Type II	Type II				Type II	
3	0.65	5.0	12	0	20	2.0	A. D.
4	0.65	1.0	13	0	20	2.0	2+
5	0.65	5.0	13	0	20	2.0	A. D.

Failure to elicit aerosol reactions with the use of type II antiserum and polysaccharide is discussed later.

TABLE IV. DESENSITIZATION FOLLOWS REACTIONS TO AEROSOL: 2 PATTERNS OF DESENSITIZATION

GP#	I.A. SENS. DOSE TYPE VI ANTI-SERUM (ML.)	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	FIRST EXPOSURE TIME (MIN.)	REACTION	INTERVAL	SECOND EXPOSURE TIME (MIN.)	REACTION	INTERVAL (MIN.)	THIRD EXPOSURE TIME (MIN.)	REACTION	INTERVAL	FOURTH EXPOSURE TIME (MIN.)	REACTION
A) DESENSITIZATION MAY FOLLOW RECOVERY FROM DYSPNEA WHILE STILL IN AEROSOL													
1	0.65	3.0	12	Severe	21 min.	8	None						
2	0.5	0.2	27	Mod.-severe	22 hr.	12	None						
B) DESENSITIZATION FOLLOWS SEVERAL DECREASINGLY INTENSE REACTIONS TO AEROSOL													
3	0.5	0.3	2½	Moderate	12 min.	2	Slt.-mod.	5	13	Slt. dysp.	5 min.	13	Very slt. dysp. (OK in fog)
4	0.65	1.0	6	Mod.-severe	24 hr.	5	Very slt.	(OK in fog in 5 min.)					
5	0.65	3.0	3	Severe	24 min.	9	Slt. dysp.	71	7	None			
6	0.65	3.0	3	Moderate	14 min.	7	Slt. dysp.	19	7	None			

TABLE V. LOCAL DESENSITIZATION BY AEROSOL WITH ONE CONCENTRATION OF POLYSACCHARIDE MAY DESENSITIZE AGAINST SEVERAL MULTIPLES OF THAT CONCENTRATION

GP#	I.A. SENS. DOSE TYPE VI ANTI-SERUM (ML.)	CONC. OF TYPE VI POLYSAC. AEROSOL USED TO DESENSITIZE (MG./ML.)	NUMBER EXPOSURES	TOTAL EXPOSURE TIME (MIN.)	REACTION		INTERVAL	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION
					FIRST EXPOSURE	LAST EXPOSURE				
1	0.5	0.3	4	37	Moderate	None	15 min.	3.0*	9	Very slt. dysp.
2	0.5	0.2	3	47	Mod.-severe	None	16 min.	3.0†	10	None
3	0.5	0.6	1	8	Slt.-mod.	None	17 min.	3.0‡	14	None

*10, †15, ‡5 multiples of original reacting and desensitizing concentrations of polysaccharide.

Experiment IV (Table IV).—Desensitization follows reactions to aerosol. Two patterns of desensitization were observed. One pattern was the desensitization which followed recovery from dyspnea while still in the aerosol. Guinea pigs Nos. 1 and 2 are examples of desensitization after recovery from single severe reactions. Desensitization of pigs Nos. 3 and 4 was completed while in the aerosol fog after their reactions had become very slight. Repeated examples of desensitization, after recovery from moderately severe dyspnea, while still in the fog have been observed in animals sensitized to and reacting to acacia aerosol.

The other pattern is the desensitization which follows two or more decreasingly intense reactions to aerosol. Guinea pigs Nos. 3, 4, 5, and 6 illustrate typical variations. Note that three were desensitized after two reactions while one required four reactions.

Experiment V (Table V).—Local desensitization with one concentration of polysaccharide may desensitize against several multiples of that concentration of hapten.

Table V illustrates this point. Note that it took a total of thirty-seven minutes in four separate exposures to desensitize one animal completely against an aerosol of a solution of 0.3 mg./ml. of type VI polysaccharide. Fifteen minutes after the last nonreacting exposure, there was a barely perceptible reaction to a polysaccharide aerosol of 3.0 mg./ml., a tenfold increase in tolerance approximately. The tolerance was increased fifteen fold by three exposures in animal No. 2 and fivefold by one exposure in the third one.

Experiment VI (Table VI).—Desensitization by aerosol is local. It does not prevent anaphylactic shock from intravenous hapten. Each of the animals was completely desensitized to the stated hapten concentration. In some acacia experiments,[6] definite systemic desensitization followed aerosol exposures of ten hours, but not four or six hours. Note that the anaphylactic reaction was weak in the last animal. Presumably, passive sensitization was fading.[5]

TABLE VI. DESENSITIZATION BY AEROSOL IS LOCAL; IT DOES NOT PREVENT SUBSEQUENT ANAPHYLACTIC SHOCK FROM INTRAVENOUS POLYSACCHARIDE

GP #	I.A. SENS. DOSE TYPE VI ANTI-SERUM (ML.)	INITIAL REACTION TO AEROSOL	CONC. OF TYPE VI POLYSAC. AEROSOL USED TO DESENSITIZE (MG./ML.)	INTERVAL (DAYS)	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.*)	REACTION	AMOUNT I.V. TYPE VI POLYSAC. (MG.)	REACTION
1	0.5	Moderate	3.0	5	3.0	None	3.0	A. D.
2	0.65	Mod.-severe	1.0	9	Not Done		1.5	A. D.
3	0.65	Severe	3.0	1	3.0	None	1.0	2+
4	0.5	Slt.-mod.	3.0	10	3.0	None	2.0	A. D.
5	0.5	Mod.-severe	3.0	11	3.0	None	1.0	1+

*Exposure time = 10 minutes.

Experiment VII (Table VII).—Local desensitization by aerosol may last at least nine days.

In each instance, local desensitization was still complete 5 to 9 days after the last desensitizing challenge with the concentrations of hapten indicated. In

two animals in Table VI, local desensitization lasted 10 and 11 days. Table VI and previous experiments[5] show that systemic passive sensitization lasts 9 days or longer.

TABLE VII. LOCAL DESENSITIZATION BY AEROSOL MAY LAST AT LEAST 9 DAYS

GP#	I.A. SENS. DOSE TYPE VI ANTI-SERUM	INITIAL REACTION TO AEROSOL	CONC. OF TYPE VI POLYSAC. AEROSOL USED TO DESENSITIZE (MG./ML.)	INTERVAL (DAYS)	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	EXPOSURE TIME (MIN.)	REACTION
1	0.5	Moderate	3.0	5	3.0	5	None
2	0.65	Severe	3.0	7	1.0	6	None
3	0.65	Moderate	3.0	7	1.0	6	None
4	0.5	Mod.-severe	3.0	9	3.0	8	None
5	0.5	Slt.-mod.	3.0	9	3.0	8	None
6	0.5	Slt.-mod.	3.0	9	3.0	8	None

POSITIVE CONTROLS

GUINEA PIG	I.A. SENS. DOSE TYPE VI ANTI-SERUM (ML.)	INTERVAL (DAYS)	CONC. OF TYPE VI POLYSAC. AEROSOL (MG./ML.)	REACTION	DESENS. TIME IN 3.0 MG./ML. AEROSOL (MIN.)	INTERVAL (MIN.)	AMOUNT I.V. TYPE VI POLYSAC. (MG.)	REACTION
1	0.5	5	3.0	Severe	50	5	3.0	A. D.
2	0.5	5	3.0	Moderate	60	10	3.0	A. D.
3	0.5	5	3.0	Slight	8	5	3.0	None
4	0.5	5	3.0	Slight	8	10	3.0	Slight
5	0.5	9	3.0	Slight	20	30	3.0	Severe
6	0.5	9	3.0	Moderate	35	15	3.0	A. D.
7	0.5	9	3.0	Moderate	35	20	3.0	A. D.
8	0.5	9	3.0	Slight	20	40	3.0	Severe

Experiment VIII (Table VIII).—Prior aerosol treatment with acacia and/or with type II polysaccharide do not prevent specific reactions to type VI polysaccharide, i.e., aerosol reactions are specific. Note that, in three instances, there were prior negative aerosol challenges, with type II polysaccharide, and that, in two animals, there had been negative prior challenges, with aerosols of both acacia and type II polysaccharide. The specific reactions to the type VI polysaccharide aerosol seemed to be unimpaired.

TABLE VIII. PRIOR AEROSOL INHALATIONS OF ACACIA AND/OR TYPE II POLYSACCHARIDE DO NOT PREVENT SPECIFIC REACTIONS TO TYPE VI POLYSACCHARIDE; AEROSOL REACTIONS ARE SPECIFIC

GP#	I.A. SENS. DOSE TYPE VI ANTI-SERUM (ML.)	CONC. NON-SPECIFIC AEROSOL (MG./ML.)	REACTION	CONC. NON-SPECIFIC AEROSOL (MG./ML.)	REACTION	CONC. SPECIFIC AEROSOL (MG./ML.)	REACTION
		Type II				*Type VI*	
1	0.65	1.0	None	—	—	3.0	Severe
2	0.65	5.0	None	—	—	3.0	Moderate
3	0.5	5.0	None	—	—	0.6	Slt.-mod.
		Acacia		*Type II*			
4	0.65	30.0	None	1.0	None	1.0	A. D.
		Type II		*Acacia*			
5	0.65	1.0	None	30.0	None	1.0	Mod.-severe

Experiment IX.—Repeated efforts were made to sensitize against type II polysaccharides alone or in combination with acacia or with type VI polysaccharide. In no instance, was the slightest suggestion of local sensitization to type II polysaccharide aerosol produced. Four different lots of type II rabbit antiserum and three different lots of type II polysaccharide were used, in varying doses of the sensitizing antiserum and concentrations of shocking type II polysaccharide aerosol. The animals which failed to react to type II aerosol invariably reacted with anaphylactic death to intravenous challenge with even weaker concentrations of polysaccharide than were used in the aerosol. The precipitin titers of the different type II antisera and polysaccharides were 1:100,000 or stronger. No explanation of the failure to sensitize to type II, in the face of ready sensitization to type VI pneumococcal polysaccharide, has been forthcoming.

DISCUSSION

The experiments reported here emphasize three aspects of desensitization which are not new; namely, the use of haptens, the use of the airway as a portal of entry for the desensitizing hapten or antigen,[7] and local versus systemic desensitization. This report does, however, present several new facets to these three broad aspects of desensitization.

These experiments show, for example, that there was no spontaneous resensitization in passively sensitized guinea pigs, after desensitization with hapten aerosol, using these relatively small doses of sensitizing antiserum. The potential advantage of desensitizing with a nonsensitizing hapten aerosol may be less obvious when excess antibody is available for resensitization of the bronchi. This is suggested by the report that actively sensitized guinea pigs desensitized by an aerosol of a whole antigen become resensitized, spontaneously, in a few days.[7] Studies of the duration of desensitization with hapten aerosols in active sensitization and in passive sensitization with homologous antiserum must wait until more suitable haptens are available. These are being sought. This delay is due to the difficulty in sensitizing guinea pigs actively with whole bacteria and immunizing them with vaccines sufficiently to obtain sera suitable for homologous serum passive sensitization.

These observations on the use of the airway as a portal of entry for a desensitizing hapten supplement those of Herxheimer,[7] who used whole antigen aerosols to desensitize. It is hoped that they will restimulate the long-dormant interest in the problems of desensitization. It is surprising that this method of local desensitization has not been exploited long before now. It has been forty years since Sewell[12] reported (on shaky grounds) that guinea pigs could be sensitized by nasal instillation and shocked by inhalations of atomized horse serum and, further, that they could be desensitized simply by living in a stable. There have been numerous other references to sensitization and shock by the inhalation of antigens.

The experiments in this report show quite clearly that the desensitization produced by the hapten aerosol is local, and not systemic. The supply of these polysaccharide haptens was too limited to attempt to measure the time required

in polysaccharide fog to absorb enough hapten to produce systemic desensitization. Feinberg[11] estimated that guinea pigs inhale about 13 gamma of 1:2,000 histamine, in 3 minutes, and about 30 gamma of Pyribenzamine while in a fog of 1 per cent Pyribenzamine, for 5 minutes. At that rate, it would take about 27 hours to inhale 1 mg. of the hapten, using a 1 mg./c.c. concentration for the aerosol. This is a borderline desensitizing amount if we assume that all of the inspired hapten was absorbed.

This concept of local desensitization is not a new one. In 1922, MacKenzie[13] and Caulfield[14] reported the successful treatment of pollen hay fever by topical nasal application of pollen extract. There are many other examples of local desensitization, which need not be reviewed. It is interesting that 30 years elapsed between MacKenzie's[13] local, specific, topical desensitization of the nose in man and Herxheimer's[7] local, specific, topical desensitization of the bronchi in guinea pigs. Perhaps, the deterrent was MacKenzie's demonstration, that local desensitization of the skin with an antigen was transient. The need to investigate the whole problem of local versus systemic sensitization and desensitization is emphasized, by these and other[10] experiments.

The results recorded in the tables seem clear cut.

It is of some interest that the duration of the desensitization was about as long as the duration of passive sensitization with heterologous serum: about ten to thirteen days.

The intensity of reactions to the same sensitizing doses of antiserum and challenging concentrations of polysaccharide varied widely.

The most violent reactions occurred most quickly, after 45 to 90 seconds in aerosol. Conversely, the mildest reactions were the slowest to appear. This conforms to the common clinical observation, that the severity of reactions to allergen injections tends to vary inversely with the delay in onset.

Since these are qualitative experiments, no effort was made to measure the amount of polysaccharide inhaled by the animals.

It would have emphasized the specificity of the desensitization if some guinea pigs had been sensitized to two antigens or haptens and had, then, been desensitized, specifically against one, without prevention of the aerosol reaction to the other. Attempts were made to sensitize against (a) type VI and type II pneumococcal polysaccharides, (b) type VI and type II polysaccharides and acacia, and (c) type VI polysaccharide and acacia. In no instance, was there evidence of multiple sensitization, with these, the only antigens tried so far. Sensitization to acacia alone is produced, with about the same frequency as to type VI polysaccharide alone. This inability to sensitize one animal to two aerosol antigens has not been explained, but should be. Experiments with different antibody-hapten systems and sensitizing schedules are planned.

A sufficient number of desensitized animals was challenged with and reacted to histamine 1:3,000 aerosol to show that the reacting mechanism was still intact.

Adequate controls seem to be found in the animals in Table I which did not react plus those in Table VIII which reacted to type VI, after failing to react to type II polysaccharide and acacia, plus those in Table II showing the threshold of concentrations reacting to type VI.

The general principles established by these experiments should be just as applicable to immunologic systems using polysaccharides from sources other than bacteria.

The pneumococcal polysaccharides are called haptens, here, with full knowledge that they may, under proper conditions, be antigenic in man and in rabbits. They are haptens because, in this type of experiment, they do not sensitize guinea pigs.

SUMMARY AND CONCLUSIONS

1. The following observations are thought to be new. They are based on studies of the effects of aerosols of pneumococcal polysaccharides, as haptens, in guinea pigs sensitized passively with specific heterologous (rabbit) antiserum.

   a. Aerosols of specific haptens elicit respiratory distress of varying intensity, from slight dyspnea to typical anaphylactic death.
   b. Reactions to inhaled hapten may differ from intravenous hapten: i.e., negative reactions to aerosol may be followed by anaphylactic death from intravenous hapten.
   c. There are definite thresholds of effective concentrations of hapten aerosols.
   d. Desensitization may be readily produced by specific hapten aerosol, in minutes.
   e. Aerosol desensitization is local: i.e., subsequent intravenous hapten will cause prompt anaphylaxis. Quantitative factors have not been studied.
   f. Aerosol desensitization may last at least nine days.
   g. Aerosol desensitization follows at least two patterns: (1) after several successive decreasingly intense respiratory reactions; and (2) recovery from respiratory distress while still in the aerosol.
   h. Local desensitization by a weak, but effective concentration of aerosol may desensitize against five to fifteen multiples of that concentration.

2. This is the third type of desensitization with bacterial polysaccharides as haptens reported from this laboratory.

3. These qualitative observations were selected for this report and are not intended to represent a comprehensive study of the problem. Others will be reported as they mature.

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ANAPHYLAXIS DUE TO TOPICAL PENICILLIN

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REPORTS in the literature dealing with anaphylaxis resulting from the topical administration of penicillin have indeed been exceedingly few.[1, 2, 4] The sites of application were limited to the maxillary antra and the bronchial tree. To our knowledge, penicillin ophthalmic ointment has not been incriminated as the direct cause of an anaphylactic reaction, and it is for this reason the following case is reported.

CASE REPORT

In 1943, during a course of heavy metal treatment for probable congenital syphilis, this 44-year-old Negro practical nurse developed a generalized pruritic rash that disappeared within two weeks after cessation of treatment.

In July, 1946, for a persistently positive serologic test for syphilis, she received a total of 4,800,000 units of penicillin over a 15-day period unattended by side effects. In February, 1953, she was given an injection of penicillin by her local physician for bronchitis, and for the remainder of the night noted tightness in the chest and wheezing. Two days later a rash appeared over her face and, although a provisional diagnosis of measles was made, no other stigmata of the disease developed.

On May 26, 1953, while on duty, she noted slight burning and redness of the right eye of several hours' duration. She applied some penicillin ophthalmic ointment containing 100,000 units of crystalline potassium penicillin G per gram to the involved eye and within seconds developed an offensive taste in her mouth. This was quickly followed by shortness of breath, a flushed appearance, cramping lower abdominal pain, and the passage of flatus and a large watery stool. There was no dysphagia, nausea, vomiting, urticaria or pruritus. There was no loss of consciousness. Asthmatic wheezes were audible throughout both lung fields. The blood pressure was 70/50 mm. Hg. Within ten minutes of the onset of the reaction, she received 0.5 ml. of a 1:1,000 aqueous solution of epinephrine subcutaneously, 64 mg. of Luminal, 0.4 mg. of atropine sulfate, and 100 mg. of Pyribenzamine.

Although the patient responded satisfactorily to this treatment within one hour, she was admitted to the hospital for further observation. General physical examination on admission to the ward revealed the following pertinent findings. There was slight injection of the right conjunctiva; there were several carious teeth. The lungs were clear to percussion and auscultation. The heart was not enlarged; the apex was in the fourth left intercostal space within the midclavicular line and there was a soft apical systolic murmur. The blood pressure was 120/70 mm. Hg and the radial pulse 98. The remainder of the physical examination was within normal limits.

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Laboratory examinations revealed the hematocrit to be 44 and the icterus index 5. The white blood count was 16,000 with 1 per cent juveniles; 85 per cent, segmented neutrophiles; 10 per cent, lymphocytes, and 4 per cent, monocytes. A sickle-cell test and urinalysis were negative.

The patient remained asymptomatic except for occasional mild respiratory wheezes and was discharged the following morning.

The family history and past history were not contributory. There was no past history of allergic reactions except as has been noted.

On Oct. 19, 1953, patch tests were done with the penicillin ophthalmic ointment and with the chemically inert ointment base alone. To the former she responded with a pruritic, erythematous wheal 1.5 cm. in diameter with several small pseudopodia, in thirty-five minutes. To the nonpenicillin-containing base there was no reaction, immediate or delayed.

COMMENT

This patient was of considerable interest in that a skin rash developed following her second exposure to parenteral penicillin. Had this latter reaction been evaluated in its true light and its importance impressed upon the patient, no doubt she would have been spared this last severe experience. It has been proposed that a patient having survived an anaphylactic reaction be provided with identification to the effect that penicillin not be administered under any circumstances.[3]

The ease with which such alarming reactions are precipitated by seemingly insignificant doses of penicillin is most distressing, and it is not beyond the sphere of reality that injudicious skin testing itself may provoke a severe or perhaps even fatal reaction.

As yet, there have been no fatalities attributed to topical penicillin. This has probably been due to relatively slower absorption from smaller doses at the site of application when compared to parenteral penicillin. The promptness in administering specific treatment to all patients, so far reported, has also aided the fortunate outcome of these cases. Undoubtedly, the case here presented would have developed more profound symptoms had treatment been withheld.

SUMMARY

A case of anaphylaxis to penicillin ophthalmic ointment in a 40-year-old female practical nurse is presented.

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Editorial

PROGRESS IN ALLERGY—A PLAN

AS AN allergist you are interested in progress in your field. This interest is not expressed solely in wishful thinking but finds an outlet in deeds and accomplishment. You believe that the public needs enlightenment, so you lecture to your patients, perhaps give a talk to a Mothers' Aid group and, possibly, write a little lay book on allergy which sells a thousand or two copies. You believe that the nonspecialist in allergy needs to know something about this subject. Therefore, you are glad to teach four or five hours of allergy in your medical school, attend an allergy clinic, give a talk, now and then, to a county medical society when the latter has run out of speakers, and perhaps participate in an occasional three-day or one-week postgraduate course. You see the need for more allergy specialists. You do what you can to participate in training such men and women in the few places available for this purpose. Every allergist knows that for the many unsolved problems in allergy steady progress in research is an absolute necessity. Some research is going on in a few places, each one doing the best it can. Many of you would like to do research and many of you have ideas, but no help is forthcoming.

In all of these endeavors, each of us is accomplishing a little but almost all of us experience a sense of frustration. To only a minute segment of the public has been carried enlightenment about allergy. The individual allergist in most places has had little success in influencing the medical school to give adequate undergraduate education in allergy. Ignorance fosters disrespect; it is not surprising, therefore, that the inadequate indoctrination of the nonspecialist has resulted in many localities in a cynical attitude toward the allergist. Greater facilities are needed for training in specialization, but again the individual allergist has been, in most instances, unable to convince medical institutions of the importance of such facilities. It is seldom that the allergist can convince the hospital authorities of the advisability of an allergy service or the need for a resident in allergy. Research could be multiplied several fold and progress accelerated if there were more cooperative planning, facilities established, and money available for support.

"Cooperative planning" is the key to the attainment of all these factors which constitute progress in allergy. Such a "cooperative" has been founded; it is already launched. It is the American Foundation for Allergic Diseases. Its aims and purposes are the accomplishment of those very deficiencies referred to above. Enlightenment of the public, enlightenment of the profession, training of specialists, better clinical facilities, and provision for an expanded research program are the attributes which will advance allergy and make you, the allergist, a more successful practitioner.

This first year of this project is being devoted primarily to public education in allergy by press, radio, feature articles, pamphlets, publicity of the

Foundation, setting up a Scientific and Educational Council which will deal with research and medical education, and some contacts with prospective philanthropic organizations. Everyone has agreed that to sell the idea to the public we must first show evidence that we have sold the idea to ourselves. It has been planned, therefore, that the cost of the first year's activities be shouldered by the allergists. The members of the Board of Trustees have been generous of their time, energy, and money. A number of other allergists have helped substantially. However, everyone must help. Every allergist should understand the plan, should contribute financially to it now, and should spread the gospel to the public and to other members of the medical profession. It is *your* plan, it is created for *you* and you must participate in it.

How can you help? Here's how:

1. Send a generous contribution to the American Foundation for Allergic Diseases. Mail it to Dr. Theodore Squire, Treasurer, 425 East Wisconsin Avenue, Milwaukee 2, Wisconsin.

2. If you are an author of a book on allergy, contribute a copy of your book to the Foundation's library. Mail it to 525 Lexington Avenue, New York 17, New York.

3. Mail a set of your reprints to the Foundation's office.

4. Distribute to your patients the folder, "Answers to Some Questions About Allergy and the Allergic Diseases" which has recently been produced by the Foundation as a service for the allergists in dealing with patients; copies can be obtained from the Foundation office. Publicize the Foundation and its purposes whenever possible.

Above all, do these things without delay. This first year is critical; your support will count twofold if given *now*.

S. M. F.

American Academy of Allergy

PRESIDENT'S ADDRESS

BEN Z. RAPPAPORT, M.D., CHICAGO, ILL.

I WANT to talk to you briefly on a subject which I believe has great significance for the future of allergy and consequently has important personal significance for everyone here—the newly established American Foundation for Allergic Diseases.

As you know, this foundation was formally incorporated in March a year ago, but its office in New York City was opened five months ago, in September, 1953. I am happy to report that this fledgling among foundations grows visibly stronger from month to month.

It was conceived under happy auspices, from the joint efforts of the Academy and the College. As many here know, there have been earlier attempts to start a Foundation for purposes similar to those of the present Foundation, but they failed, mainly because of too limited sponsorship. In this project, the Academy and the College have cooperated fully to assure success. Working together, we can establish the American Foundation for Allergic Diseases on a sound basis and can develop a far-reaching medical and scientific program which will better achieve the purposes of the Academy and the College.

Necessarily, the Foundation has had to begin in a small way, since it was financed initially by contributions from a comparatively small group composed of the medical trustees of the Foundation and a few others. For the past five months the staff has consisted of three persons: an office secretary, a writer, and a fund-raiser. The last two are assigned from Reuel Estill and Company, who have been retained as public relations and fund-raising consultants. The writer, Miss Helen Denman, comes to us after having served four years with the Arthritis and Rheumatism Foundation which is only five years older than the Foundation for Allergic Diseases. She did an outstanding job of writing for this organization and is beginning to do a similarly good job for us. The fund-raiser was assigned to us without charge by Mr. Estill, and all are working under Mr. Estill's close supervision.

Now what has the Foundation accomplished in this first five months? The initial fund of about $12,000—resulting from the investment of faith and hope and conviction by a comparative few—has been increased by $25,740, making a total fund of more than $37,000 as of January 22. This increase represents the contributions and pledges of about 200 members of the Academy and the College, plus grants from the Academy and the College, and a few lay contributions that came unsolicited. When we go to the public for support of the Foundation, as we must before very long, no small part of our acceptance will be the sincerity evidenced by the fact that the allergists themselves were so completely convinced

of the need for a Foundation for Allergic Diseases that they contributed substantially to aid in its establishment.

You can see, however, that percentage-wise the response from the memberships has not been impressive. It can, and should, be much better. Any in the audience who have been planning to make a contribution and have somehow overlooked or postponed the decision, still have the opportunity to do so. The Foundation office will be very happy to receive your check.

As you know from the preliminary announcement of the Foundation, it was decided that this first year should be spent in informing the public on the meaning and the importance of the allergic diseases. We have a different problem from any other foundation and in some ways a more difficult one. The word "allergy" has been popularized to the extent that it has all but lost its original medical meaning. We must reinvest allergy with its true significance. Not only that; we have to dispel some popular misconceptions: that allergy is just an "idea," a notion on the part of the allergic person, and that the patient is a type of malingerer.

Before we approach the public for support of the Foundation, it is important that we realistically impress the public with the seriousness of allergy, especially asthma, as a distressing and important illness which, while it may not kill, may be totally incapacitating, so that the patient becomes a burden to himself, his family, and the community. We have to tell the public what a tremendous amount of allergy exists in the population, and point out the need for greater facilities for treatment throughout the country. This, briefly, is the objective of the Foundation's public relations program this first year, and it has to be done on a national scale, using as many media for reaching the public as can be handled by our limited task force.

We have started by using the medium of the press. Since our initial announcement of the Foundation, one news feature has been sent out nationally to leading metropolitan dailies, the Associated and United Press, the International News Service, the various syndicate services, and the weekly and monthly periodicals, especially those catering to women's interests. The subject of this news story was reactions to penicillin. We are using this material not only to disseminate valuable information about allergy and the allergic diseases but to establish the Foundation in the public mind as a source of information. This news release has been well received and I am told that the Foundation headquarters has many newspaper clippings.

There are many voices seeking the public attention—on behalf of cancer, heart disease, infantile paralysis, multiple sclerosis, arthritis—and we shall have to tell the story of allergy over and over for a long time to produce the necessary impact on public attention and opinion, but we have made a good beginning.

Whenever a national news release is sent out, the Foundation headquarters receives a flood of inquiries by letter and telephone from desperate people, describing symptoms, seeking relief. Of course, it is impossible to give these

unfortunates what they ask. To give them some understanding of their problem and what they should do about it—to safeguard them from quack claims and questionable remedies—a comprehensive and easily understood leaflet has been prepared in the form of Questions and Answers about Allergy, which will be in print by the middle of this month. This folder will be available to members of the Academy and the College for use with patients, can be distributed in clinics, in schools, to public health nurses, Parent-Teacher Associations, women's clubs, and any other groups. The unit cost is about two cents a copy so that the charge for several hundred copies will be small.

This is the first of a series of informational pamphlets which the Foundation will produce in the course of time as specific need becomes apparent. In some cases reprints of existing pamphlets written by Foundation trustees may be used.

We are seeking to reach the general public with much of this educational and interpretive material. But there is what might be called a "captive" audience for information on allergy.

You will recall that along with the Foundation's appeal for your contribution there came a request for names of influential laymen in your community who might contribute to the Foundation either money or leadership, or both. Many of you have sent in names and have offered to help enlist this lay support. Many of you have yet to be heard from. But I am sure that you will respond as you realize how important lay support is in the growth and development of the Foundation. We know, from the example of the Foundation for Infantile Paralysis, the American Cancer Society, the Arthritis and Rheumatism Foundation, and other organizations similar to ours, that there has to be a combination of professional knowledge with lay interest and enthusiasm if the American Foundation for Allergic Diseases is to grow and realize its vast opportunities. We doctors alone cannot produce the necessary funds from the public; we need the partnership of our patients.

So this is what I mean by a "captive" audience; it is the men and women in every community who have a personal reason for being interested in allergy and are already convinced of the need to do something about the allergic diseases; who will spread information about the importance of allergy, especially asthma, the inadequacy of research, the limited opportunities for specialized training, and the lack of facilities for patient care. These people will contribute, and will organize their communities to contribute, to the Foundation's program. Many of these individuals are patients of yours. All those who are victims of allergic diseases, their parents, relatives, friends, are naturally deeply concerned with the cause of the Foundation. They are our immediate audience.

I want to emphasize that no doctor need feel any hesitancy about asking a patient to support the Foundation. In the first place, you are not asking that patient to help you. You are asking him to help himself and countless others like him. Any man or woman whom you have relieved of the distress of an allergic condition, or whose child has been relieved and restored to good health, will deem it a privilege to give his support to the Foundation, at your suggestion.

You are going to be asked to go over your lists of patients, past and present, and to select those who, because of their capacity to give and the service you have rendered them, should become charter supporters of the Foundation. It has been through their physicians that many of the leading men in this country have become outstanding philanthropists.

To enable these laymen to be really informed spokesmen for the cause of allergy, the Foundation plans to issue a bulletin in popular form, which will carry information about the allergic diseases, new developments in therapy, new clinics, and so on. But this will have to come later when we have built up a sufficiently large lay group. I hope that all those who have not sent in the names of influential laymen, will now be impelled to do so. In the next few months we should augment our Board of Trustees with at least an equal number of laymen who will be as outstanding in business, industry and the professions as our medical trustees, and as convinced of the need to do something about allergy.

While these various public relations and fund-raising activities which I have briefly sketched are going on, the fundamental task of shaping the Foundation's medical program must be proceeding. As you will recall from the preliminary announcement, the Foundation has four broad objectives: the stimulation of basic research in the causes of allergic diseases; the deepening of professional training in the medical schools, with an effort to induce more competent young physicians to specialize in allergy; the extension of hospital and clinic facilities for patient care; and, finally, the development of home care and ambulatory treatment for the medically indigent and those unable to finance long-drawn-out illness.

Just how these objectives will be approached—for instance, by what means the Foundation will stimulate allergy research, how it will go about producing more opportunities for specializing in allergy, how it will induce many more general hospitals to put in allergy clinics—is the responsibility of a specially constituted Scientific and Medical Council acting in an advisory capacity. As the Council and its duties have been envisaged, it will be composed one-third of basic scientists; one-third of medical educators; and one-third of clinicians equally divided between the Academy and the College. According to the Foundation's bylaws, the selection and enlistment of these men is the task of the Program Committee, which consists of Murray Peshkin, Harry Rogers, John Sheldon, William Sherman, Warrick Thomas and Samuel Feinberg, Chairman. They have been hard at work for the past two months and we are going to have some highly interesting news soon.

I said at the outset that the development of the Foundation for Allergic Diseases is of important personal significance to each of us. No one here needs to be told how limited is the research in allergy. In the period from 1946 through 1951, according to the National Research Council, allergic diseases ranked 26th in a list of 29 diseases which received research support from government and private sources. Last year 99 grants were made by government and private sources for allergy studies as compared with 2,470 public and private grants for cancer investigation. Total expenditures for allergy amounted to $800,000 as

compared with 27 million for cancer. No wonder skilled investigators are going into other fields than allergy!

This disparity must be reduced. The Foundation, through its Scientific and Medical Council, will work for a greatly increased share of research funds.

We all recognize that the man intending to go into allergy needs education that is intensive and yet along the broadest lines. He should be an internist or pediatrician with sound clinical experience who has also a basic knowledge of immunology. He should have the special skills necessary to detect sensitization and its causes. Yet we know how poorly organized and inadequate is the instruction in allergy offered by a great many medical schools, and how few are the opportunities for residencies and intensive clinic experience which will equip a well-grounded specialist. Centers should be available for well-organized post-graduate study where men practising allergy may register for additional training as their need arises.

When you consider that there are, conservatively estimated, about 7 million victims of allergic diseases in the United States and, at the most, less than 2,000 qualified allergy specialists, you can see how urgent is this problem of adequate professional training and of more qualified men. Training vies with research in priority on the Foundation's program.

From the standpoint of the practitioner, the lack of facilities for patient care is probably the most immediate problem. Here in your office is a patient with asthma who requires immediate hospitalization and no general hospital in your community will accept him because he requires too specialized care for too long a time. Outside the large metropolitan centers there are few clinics staffed by allergists for the care of the poor.

Here is where the lay committees to be organized by the Foundation will function with energy and enthusiasm. The experience of other health and medical foundations has been that the development of local resources for care of the suffering individuals in the community makes an irresistible appeal to the layman and dramatizes the objectives of the Foundation as neither research nor professional training can, fundamental as these objectives are.

This program of the Foundation is very broad and it will take time to bring it to fruition. It will also take the financial and the moral support of every man in our two societies. But it will bring rich rewards to the field of allergy and to its practitioners.

ALLERGY IN OUR TIME

ADDRESS OF THE PRESIDENT OF THE AMERICAN COLLEGE OF ALLERGISTS
M. MURRAY PESHKIN, M.D., NEW YORK, N. Y.

AT THE turn of the century Richet introduced the term "anaphylaxis" to describe the phenomenon of hypersensitiveness observed in animals, the counterpart of which was the classical syndrome of "serum-sickness" in man, described by von Pirquet and Schick, and von Pirquet's term "allergy" as applied to any type of human hypersensitiveness. Today the term "allergy" is employed universally.

The antigen-antibody theory of allergy advanced by von Pirquet and Schick in 1907 still prevails despite the current knowledge of collagen diseases and the histamine theory, the use of antihistaminic preparations and adrenocorticotropic hormones. There is a need to pay attention to the insidiously creeping paralysis of empiricism in allergy thinking and treatment. This empiricism has been speeded up with the advent of the "miracle drugs" which have created the practice of employing the easiest way out of the dilemma of treatment for the intractable and even average allergy patient. This situation requires correction if we hope to check a slow deterioration of the art and science of the practice of allergy.

From an immunologic and therapeutic viewpoint, specific allergenic treatment remains the most reliable and effective method for relieving the large majority of hay fever and asthma sufferers. In recent times the allergist has become aware of the psychologic aspects of allergy and is already treating the allergic patient as a total human being rather than a person with merely an allergic disorder.

From an organizational standpoint we are fortunate to have our interests looked after by two national societies, the Academy and the College. The regional and local allergy societies have a special usefulness when functioning at the local or regional level. In 1951, the First International Congress of Allergy was held in Zurich, Switzerland, by the International Association of Allergology, its president, Dr. Fred W. Wittich and president-elect, Dr. Samuel M. Feinberg.

About March, 1953, the American Foundation for Allergic Diseases was incorporated in the State of New York. This foundation is sponsored by both national allergy societies. It is a lay organization with many sound functions. It will ethically present the subject of allergy to the public, defend allergy, and, when necessary, even come to the defense of allergists, promote social and scientific research, and contribute to the rehabilitation of the "chronic asthmatic cripple," especially the intractable asthmatic child not responding to modern allergy practice in his own community. This is only a part of the Foundation's

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program, which is being handled by an efficient medical public relations organization along with the teamwork of your representative members on the Board of Trustees. The Foundation is the only acceptable link between the allergists and the public. The initial success of launching the Foundation is the responsibility of the allergists. The allergists for their own protection are obligated to nurse the Foundation during its first year of operation and its life-sustaining nutrient is your voluntary financial support. This is a worth-while investment. The president of the Foundation is a conscientious, diligent, untiring and intelligent gentleman. He is doing a sound job. Our faith and trust in his leadership are well founded. It is a privilege to work with our good friend, Dr. Horace S. Baldwin.

There is a need to encourage the establishment of a separate, independent Department of Allergy in the hospitals of this country. The director of such a department should have equal rank with the recognized specialties and should be designated the attending allergist. The attainment of this goal is largely dependent on allergy acquiring the status of an independent specialty through an American Board of Allergy. A natural and subsequent goal is the establishment of a separate department of allergy in our medical schools. It may be difficult to hold the attention of sympathetic ears if allergists fail to show an unselfish interest in preserving the specialty of allergy so that the public—the patients—the important focus of our attention—is properly and humanely treated by competent allergists. There is also an urgent need to induce qualified young physicians to become allergists. In recent years the latter have become a progressively dwindling group. For allergy this is a realistic alarm and an ugly storm signal.

This brings us to a consideration of the vital topic of "Allergy Certification." Allergy as a specialty was first challenged in 1939 when the allergists became a subspecialty of the American Board of Internal Medicine. The picture was changed a few years ago when allergy was further divided to become a subspecialty of the American Board of Pediatrics and, as a consequence, the grandchild of the American Board of Internal Medicine. There are reasons to suspect great-grandchildren in the offing.

Sensing that unity and strength of purpose among allergists were primary requisites to hold the allergists together, the two national allergy societies, represented by a Joint Committee on Certification, have, for the last six years, unsuccessfully appealed to the Advisory Board for Medical Specialties for an autonomous American Board of Allergy. These denials pose a serious threat to the future development if not the survival of allergy as it is practiced today.

Last September, however, an autonomous American Board of Allergy seemed to have emerged from its certification vacuum and hopes for obtaining recognition almost reached the highest realistic peaks. It should be a source of concern to all allergists to know that one month later in October, the allergists of America suffered a most unexpected setback, a notorious painful rejection by the American Board of Pediatrics. This then is the record. The failure of the Joint Certification Committee, of which Dr. George Piness was the able and

untiring chairman, to deliver positive results is an understandably keen disappointment for all of us. Yet, the matter cannot be allowed to rest.

In organizational allergy where the overwhelming forces of right, representing both national allergy societies, and the tiny forces of opposition, represented by the dissident pediatric allergists, are aligned for survival, there is no room for seeking possible selfish advantages, for maneuvering in the sidelines, or for the hazardous game of neutralism. The choice is between cooperation with the forces of freedom for allergy certification and the path of slavish subcertification. The American Academy of Allergy and the American College of Allergists long ago chose freedom for allergists. We have committed ourselves to an autonomous American Board of Allergy within the framework of the Advisory Board for Medical Specialties. We remain committed. United, we shall carry on the crusade, holding firm to the viewpoint that the allergists of this country are mature enough to be able to see through any plan endangering the establishment of an autonomous board of allergy.

The immediate objective is to press for hearings between the Joint Committee on Certification and the Pediatric Allergists and their American Board of Pediatrics, so that the issues involved may be carefully reviewed and studied. It is vital that all allergists remain loyal and self-effacing for the cause of allergy and for the allergists of tomorrow. It is to be earnestly hoped that the pediatric allergists who have faltered in their support of the major issue—an autonomous board of allergy—will reverse their course and do all in their power to help bring about the wishes of the vast majority of allergists as represented by the Academy and the College.

We feel that the hour is getting late. And the allergists of America in unison may request a definitive response to "When will this confusion end?"

HISTORIAN'S REPORT

The Tenth Annual Meeting of the Academy was held at the Shamrock Hotel in Houston, Texas, on Monday, Tuesday, and Wednesday, Feb. 1, 2, and 3, 1954. This was the first meeting of the Academy in the Deep South, and those in attendance from other parts of the continent thoroughly enjoyed the Southern Hospitality extended by the allergists of Texas, and the warm and sunny weather which prevailed.

The officers and members of various committees began to arrive on Thursday, January 28. The Credentials Committee met all day Friday. The Budget Committee and the International Committee held shorter meetings on the same day. On Saturday the Executive Committee met from 9:30 A.M. to 6:00 P.M. Meetings were held on Sunday by the Committee on Education, the Research Council, and the Committee for Certification in Allergy. Further meetings were held after the scientific sessions began by the Public Relations Committee, the Editorial Board of the Journal of Allergy and its Liaison Committee, the Committee on New Drugs, and the Committee on Insect Allergens. At noon on Wednesday, the Executive Committee of the Academy held its usual second meeting, at which the new President, Dr. John M. Sheldon, presided and which was attended by the newly elected officers and members: Dr. Joseph Harkavy, Dr. Francis C. Lowell, Dr. Max Samter, Dr. Homer A. Howes, and Dr. Clarence Bernstein. Leaving the committee as their terms expired were Dr. Horace S. Baldwin, Past-President, Dr. Frank Perlman, and Dr. Howard Osgood.

To take advantage of the presence of men from all parts of the country, meetings were also held of the Executive Committee and of the Board of Trustees of the American Foundation for Allergic Diseases.

Three postgraduate courses were held under the auspices of the Committee on Education preceding the scientific sessions. Baylor University College of Medicine made available its lecture rooms and laboratories for these courses. The Academy is appreciative of this courtesy and hospitality.

The first course, on Friday and Saturday, January 29 and 30, under the chairmanship of Dr. Walter L. Winkenwerder, consisted of a Symposium on ACTH and Cortisone, a Clinic on Chronic Allergic Vasomotor Rhinitis, a Visiting Allergy Clinic and a Symposium on Dermatologic Allergy.

The second course on Saturday and Sunday, under the chairmanship of Dr. S. M. Feinberg, consisted of advanced seminars on Histamine and Hypersensitivity and on the Pattern of Response of Individual Species in Hypersensitivity.

Mr. O. C. Durham, chairman of the Pollen and Mold Committee, Dr. Clyde M. Christensen, University of Minnesota, and Dr. Marie Morrow, University

of Texas, conducted the third course, which was a practical one on the Identification, Distribution, and Clinical Significance of Allergenic Molds and Pollens.

The scientific sessions, held for two and one-half days beginning at 9:15 A.M. on Monday, February 1, were very well attended. The program was one of the best in which the Members and Fellows and guests of the Academy have participated. A good balance was achieved between clinical topics and experimental work. The various papers were conveniently grouped so that those covering similar subjects were presented in sequence.

The major lectures were: "The Development of Immunohematologic Concepts" by Dr. William Dameshek, Director of the Blood Research Laboratory, New England Center Hospital and Clinical Professor of Medicine, Tufts Medical College, Boston, and "The Dynamics of the Immune Response" by Dr. Frank J. Dixon, Chairman of the Department of Pathology, University of Pittsburgh. Dr. Walter S. Burrage of the Harvard Medical School and the Massachusetts General Hospital presented a moving picture showing the changes under the microscope in the arterioles, venules, and capillaries of the lungs of living rabbits before and after the intravenous injection of epinephrine and histamine.

The annual business meeting of the Academy was held on Tuesday afternoon, February 2. At this meeting Dr. George Piness reported for the Committee on Certification in Allergy. Through the close cooperation and unity of purpose of the American Academy of Allergy and the American College of Allergists, the prospects for a separate Board of Allergy are considerably improved. Dr. Piness stated that the question of a separate board has been given sympathetic consideration by the Advisory Board of Medical Specialties and by the American Board of Internal Medicine. It may be given further consideration by the American Board of Pediatrics and by the American Academy of Pediatrics. Dr. Piness expressed the opinion that next year he may have a more definite report to make. The following resolutions by Dr. Piness and by Dr. Murray Peshkin, respectively, were passed unanimously.

By Dr. Piness:

BE IT HERE AND NOW RESOLVED, that this Committee continue to cooperate with a similar Committee of The American College of Allergists in all its activities pertaining to and leading to an American Board of Allergy.

By Dr. Peshkin:

BE IT HERE AND NOW RESOLVED, that this Committee be given a vote of confidence that it truly represents the desires of the voting Fellows of the Academy in its devotion to establish an American Board of Allergy, and,

BE IT FURTHER RESOLVED HERE AND NOW, that the Committee on Certification be instructed to continue vigorously its endeavors to establish an American Board of Allergy within the framework of organized medicine based on the principles expressed in this and in other reports of the last year.

Certain minor amendments to the Constitution were presented by the Committee on the Constitution and Bylaws after approval by the Executive Committee. One amendment clarified the requirement that for Fellowship a candidate must fulfill "at least two of the three criteria" stated in Article II, Section 2, of the Constitution. The other amendment provided that the President-elect shall automatically assume the Presidency upon retirement of the incumbent President. This succession, through inadvertence, had not been specifically stated in the Constitution at the time the office of President-elect was created. The President-elect, therefore, is elected annually, not the President. The President-elect is a member of the Executive Committee and is in constant and close touch with the President. Both amendments were passed unanimously.

Dr. S. M. Feinberg, President-elect of the International Association of Allergology and member of the Academy's International Committee, reported on the activities of this Association and on the program and plans for the next International Congress on Allergology to be held in Rio de Janeiro, Brazil, in 1955. The date for this Congress has not been definitely determined. The desires and convenience of men from the Western Hemisphere as well as those from Europe, the Near East, Africa, Australia, and the Orient have to be taken into consideration. Dr. Feinberg requested those present at the business meeting to fill out a vote-sheet as to (a) whether or not they might attend the Congress and (b) what month would be their first choice for the date. This vote indicated that about 50 per cent of those present would consider going to Rio de Janeiro. The month of November was given the greatest number of votes as being the most acceptable. November will be late spring in Brazil. Dr. Feinberg also extended an invitation to those present to submit papers to be read at the Congress. There will be at least five days of scientific sessions. Certain general sessions will be held for all attending the Congress. At other times, several simultaneous sessions will be held, at which papers covering similar fields will be grouped.

In recognition of his outstanding contributions in the field of allergy and of his many years of devoted service to the Academy as Editor of the JOURNAL OF ALLERGY, as an officer of the Academy and as an effective member of numerous committees, Dr. Harry L. Alexander of St. Louis was unanimously elected an Honorary Fellow of the Academy.

The following officers were elected for the year 1954-1955:

President-Elect: Dr. Stanley F. Hampton
Vice-President: Dr. Joseph Harkavy
Secretary: Dr. Francis C. Lowell
Treasurer: Dr. Max Samter
Historian: Dr. Homer A. Howes
Member of Executive Committee: Dr. Clarence Bernstein

Dr. John M. Sheldon, before the close of the business meeting, assumed the Presidency for the coming year, having been elected President-elect last year. A unanimous vote of appreciation was extended to Dr. Ben Z. Rappa-

port, retiring President, for his highly successful administration and for his untiring work and efforts on behalf of the Academy. Dr. Rappaport remains on the Executive Committee as Past President for the next two years.

The President's Luncheon for all members and guests was held on Tuesday, February 2. In his address, President Rappaport described the development and final organization of the American Foundation for Allergic Diseases. This Foundation has now been fully established, with the support of both national allergy societies. The Board of Trustees has been formed, at present consisting of prominent physicians closely connected with allergy and drawn equally from both national allergy societies. Prominent laymen from all parts of the country will be added to this Board, thus giving it a broad basis of interests.

Dr. Rappaport said that at present efforts must be made to publicize the extent of allergic diseases and the need for large financial support to promote research in allergy and for the better care of persons disabled with these widespread diseases. The activities of the Foundation will be at first directed toward the formation of local organizations of laymen and physicians as essential preliminaries to the later collection of funds. Although a generous amount (over $30,000) has already been contributed by the Academy and the College and by individual physicians, considerable more money will be needed for this first year. Dr. Rappaport asked that each of us contribute in large or small amounts. The Foundation Trustees are as anxious to have five-, ten-, and twenty-dollar gifts (tax deductible) as those of larger amounts.

On Sunday evening, the Texas allergists entertained the members and guests in the assembly hall of the Prudential Life Insurance Building in Houston, within walking distance of the Shamrock Hotel. The Committee on Local Arrangements, under the chairmanship of Dr. Homer E. Prince of Houston, made this social hour the occasion for presenting to President Rappaport a Texas Ten Gallon Hat as a memento of the meeting.

Monday evening members and guests were transported by bus twenty miles south of Houston to the San Jacinto Inn, where they were entertained through the courtesy of the Schering Corporation. This was followed by a delicious Texas dinner. The visitors were astonished upon arrival to find the Battleship Texas anchored in the ship canal practically in the front yard of the Inn. Instead of permitting the battleship to be scrapped after the Pearl Harbor tragedy, the citizens of Texas raised sufficient funds to rehabilitate the ship and to bring it home for a permanent monument.

The ladies present during the convention period were welcomed by the Hostess Committee of which Mrs. Ben Z. Rappaport was Honorary Chairman and Mrs. Homer E. Prince, Chairman. A tea Sunday afternoon, a boat trip down the Houston Ship Canal to the San Jacinto Battlefield on Monday afternoon, and a luncheon on Tuesday at the Shamrock Hotel were among the entertainments offered. Preceding the luncheon, courtesies were extended by the Marcelle Cosmetics Company.

The annual meeting closed at noon on Wednesday, February 3. The Members and Fellows of the Academy, their wives, and guests started on their

several journeys with gratefulness to their Texas hosts and hostesses for a most delightful visit and with pleasant memories of a fine and prosperous city.

The total registration at the meeting was 394. Of these, 111 were Fellows, 96 were Members, 97 were guests, and 90 were commercial exhibitors. There were 35 Commercial Exhibits and 7 Scientific Exhibits.

Annual meetings of the Academy will be held on the following dates and at these cities:

1955, Feb. 7, 8, 9: New York City, Statler Hotel
1956, Feb. 6, 7, 8: St. Louis, Chase Hotel
1957, Feb. 4, 5, 6: Los Angeles, Statler Hotel

A list of candidates who were elected to membership at the business meeting of the Academy on Tuesday, February 2, 1954, follows:

TO HONORARY FELLOWSHIP

Harry L. Alexander	Mauricio Rocha e Silva

TO FELLOWSHIP

Theodore H. Aaron	Abe L. Aaronson
Robert D. Berke	Vincent J. Derbes
Alan R. Feinberg	John D. L. FitzGerald
Ralph W. Grover	Robert G. Lovell
Kenneth P. Mathews	David Merksamer
George A. Peck	Bernard B. Siegel
Jerome J. Sievers	Miguel A. Solari
Boen Swinny	Eugene H. Walzer

TO AFFILIATE FELLOWSHIP

Jose F. Dumm	Buenos Aires
Beningo R. Garat	Buenos Aires
Israel Glazer	Tel-Aviv, Israel
Amadeo Vicente Mastellari	Panama
Leslie A. I. Maxwell	Melbourne, Australia
Charles Sutherland	Melbourne, Australia

TO MEMBERSHIP

George Babcock	Leon Bentolila
Martin Brewda	Norman W. Brey
William J. Briggs	Halla Brown
Haddon M. Carryer	Horace B. Chapin
Archie Cun-Ming	Monroe Cohen
Edward M. Di Tolla	Clyde F. Elkins
Milton Engel	Lawrence N. Ettelson
D. Edward Frank	V. E. Friedewald
Harvey E. Gaynes	Saul Grossman
Salmon R. Halpern	Morton L. Hammond
Henrietta Hartley	Conrado B. Icasiano
John W. Irwin	Frederick Kessler

George R. Laub
Emmett C. Mathews
Robert P. McCombs
Francisco J. Menendez
Lloyd S. Nelson
Gustavus A. Peters
Norman A. Pokorny
Harold Rosenberg
Gertrude Sobel
John L. Thompson
Irving I. Weintraub
Lowry C. Macbeth
Carl D. Marsh
James A. McLean
Charles F. Mohr
Beverly C. Payne
Horace Pettit
Robert W. Raymond
David Shapiro
Robert A. Stier
Richard R. Weigler
William I. Weiss

HOWARD OSGOOD, Historian.

REPORT OF COMMITTEE ON NEW DRUGS

A NEW antihistaminic vibazine (Pfizer Compound No. 4445) was studied by the following members of the Committee on New Drugs: Drs. Murray Albert, Clarence Bernstein, Theodore Bernstein, Robert Chait, Vincent Derbes, Sidney Friedlaender, Harold Friedman, Frank Joyce, Edmund Keeney, Dean Moffat, Milton Mosko, Irving Schiller, Aaron Spielman, Walter Winkenwerder, and Carl Arbesman.

The results were tabulated on special IBM cards, and various cross relationships were determined. This drug was given to 545 patients, and the data obtained are listed in Table I. Approximately one-third of the patients with either seasonal or perennial allergic rhinitis had adequate benefit with Compound No. 4445. Like other antihistamines, however, the symptoms of rhinorrhea and sneezing were more adequately controlled than that of nasal occlusion. This substance was ineffective in bronchial asthma and only relatively beneficial in urticaria.

TABLE I. PFIZER NO. 4445 (545 PATIENTS)

CONDITION	NUMBER OF PATIENTS	PATIENTS IMPROVED* (%)	
Seasonal allergic rhinitis	395†		29.1
Perennial allergic rhinitis	66		34.7
Seasonal allergic rhinitis with assoc. bronchial asthma	47	Nasal	29.6
		Bronchial	3.0
Bronchial asthma (all types)	25		8.0
Urticaria	12		45.0

*Only those patients having 50 per cent or more relief of all symptoms were classified as improved.

†An additional 50 patients not processed with IBM cards. 32 per cent had satisfactory relief.

This drug, when effective, gave relief within 30 minutes in 85 per cent of the patients (Table II). The action of the drug, however, lasted only one to four hours in 83 per cent of the cases who responded well. Beneficial results for eight or more hours was recorded in 9 per cent (Table III).

TABLE II. PFIZER NO. 4445 TIME OF ACTION (383 PATIENTS)

LENGTH OF TIME TO BECOME EFFECTIVE (MIN.)	NUMBER OF CASES	(%)
15 to 30	326	(85)
45 to 60	38	(10)
90 plus	19	(5)

TABLE III. PFIZER NO. 4445 DURATION OF ACTION (346 PATIENTS)

LENGTH OF TIME EFFECTIVE (HOURS)	NUMBER OF CASES	(%)
1-2	155	(44)
3-4	134	(39)
5-6	26	(8)
8 or more	31	(9)

Compound No. 4445 had a very low incidence of side effects. Only 6.0 per cent of patients had moderate to severe side effects. The side effects encountered were similar to those produced by other antihistamines. Of sixty-eight children ten years of age or under, not one reported any side effects from this drug.

The age of the patient did not appear to be a factor in the degree of relief obtained with this substance. From the data obtained, however, those patients with symptoms of allergic rhinitis for less than one year seemed to respond best with this compound.

TABLE IV. PFIZER NO. 4445 INCIDENCE OF SIDE EFFECTS

Total number of patients	547	
Total number of side effects	139	(25.4 per cent)
Total number of moderate to severe side effects	38	(7.0 per cent)
Number of patients with moderate to severe side effects	33	(6.0 per cent)

CONCLUSIONS

Compound No. 4445 (Pfizer) is only a fairly active antihistaminic drug. The low incidence of side effects, however, makes it desirable for those patients who cannot tolerate the more potent antihistamines. Like other antihistamines, its chief value is for the symptomatic relief of rhinorrhea and sneezing in allergic rhinitis and pruritus in urticaria. It is ineffectual in bronchial asthma. The time of action and its duration of effectiveness have no advantage over similar substances already available.

Carl E. Arbesman, Chairman,
Committee on New Drugs,
American Academy of Allergy

Obituary

WARFIELD THEOBALD LONGCOPE, one of the founders of the Society for the Study of Asthma and Allied Conditions, died at the age of seventy-six at his summer home at Lee, Massachusetts. Dr. Longcope came of a family long interested in medicine, and he represented the fifth member to hold a professorship in the field of medicine in American universities. Born on March 29, 1877, son of George Von S. Longcope and Ruth Theobold Longcope, Dr. Longcope was graduated from the Johns Hopkins University in 1897, and from the Medical School in 1901; thereafter, instead of following the usual pathway of internship and residency training, he elected basic training in pathology at the Pennsylvania Hospital, where he later became director of the Aver Clinical Laboratory (1904-1911).

Interest in clinical medicine was soon reflected in his appointment in 1909 as Assistant Professor of Medicine at the University of Pennsylvania, and later in 1914 in his appointment as Bard Professor of the Practice of Medicine at Columbia University and, also, as Director of Medical Services at the Presbyterian Hospital, New York City, a dual post he held until 1922. During World War I, he served in the Surgeon General's office, and later as Consultant with the rank of Colonel in the American Expeditionary Forces. In 1922, he was called to his alma mater and was appointed Professor of Medicine at the Johns Hopkins University, and Physician-in-Chief to the Johns Hopkins Hospital, appointments which he maintained with marked distinction until his retirement at the age of sixty-nine in 1946.

Dr. Longcope's interest in medicine was broad and basic. His studies and publications correlating experimental, pathologic, and clinical manifestations of disease were many and varied. His description of syphilitic aortitis in 1914 clearly defined the pathologic and clinical features of this lesion. His early interests also concerned the experimental and clinical aspects of hypersensitivity, which led to his participation in the founding of the Society for the Study of Asthma and Allied Conditions in 1924. His writings and publications, to mention a few, dealt with serum disease, glomerular nephritis, Boeck's sarcoid, Hodgkin's disease, atypical pneumonia, and allergic manifestations to sulfa drugs. During World War II, he and his staff carried on extensive studies on the use of BAL in metallic poisoning.

Membership in many medical societies, many academic honors, and positions of administrative responsibility were natural corollaries to his high abilities as a teacher and investigator. Dr. Longcope was a member of the American Association of Physicians, the Society of Clinical Investigation, the American Association for Advancement of Science, the National Academy of

Science, the American College of Physicians, the Harvey Society, and many others. He was an Honorary Fellow of the Royal Society of Medicine of London, an Honorary Member of the Societe des Hospiteaux de Paris, and Honorary Fellow of the Scandinavian Congress for Internal Medicine. In 1934, he was elected a member of the Board of Scientific Directors of the Rockefeller Institute for Medical Research, and in 1939 became its president. He was president of the American Association of Physicians in 1945, and in 1948, the association in recognition of his many contributions to the field of medicine awarded him the Kober Medal.

The mere recording of Dr. Longcope's contributions to medicine, his membership in many scientific societies, and the recognition of his distinctive abilities by his associates do not adequately portray his vital day-to-day activity as administrator of his department of Medicine, or as a teacher on the wards with his students and members of his immediate staff. To the latter he was particularly devoted, and no one could have been more interested in the education of younger men, which showed itself not only in the hospital environment but also in his home, where with his charming wife, Janet Longcope, his staff and students were always welcome and often entertained. His ideals were high, and his interest in the experimental aspects of disease did not detract from this attitude on the wards where the patient's welfare was always his first consideration. His curiosity as a teacher was most stimulating and also quickly aroused, and he would devote the deepest interest to a seemingly insignificant detail to which his attention was called without, however, displaying a patronizing attitude which might embarrass the neophyte though one knew later he was perfectly familiar with the point under discussion. His role as teacher was not to dampen but to stimulate the curiosity and enthusiasm of his students and staff, as he was always a student to the end of his career. Medicine lost a great physician in every sense of the term and those who were fortunate to be included in his immediate circle lost a stimulating teacher and a staunch friend.

Walter L. Winkenwerder, M.D.

Announcement

Residency in Allergy

Northwestern University Medical School offers a new Residency in Allergy in connection with the new Veterans Administration Research Hospital. The resident will be in charge of a service at the hospital and spend one-half of his time at the Medical School in clinics and research. This residency is in addition to the Fellowship in Allergy which will continue to be offered at Northwestern. The service will begin July 1, 1954. Applications should be made to Dr. Samuel M. Feinberg, 303 East Chicago Avenue, Chicago 11, Ill.